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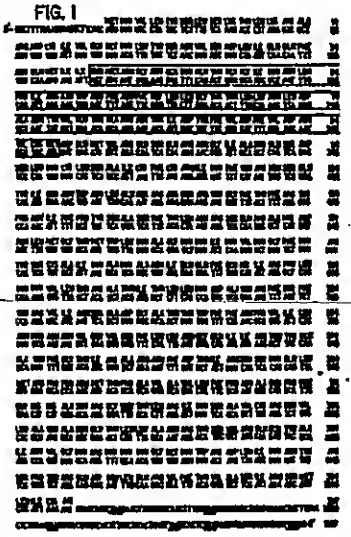
(54) The use of rotavirus nucleocapsid protein VP6 in vaccine compositions.

(57) New immunological carrier complexes are provided utilizing the VP6 polypeptide from rotavirus as the carrier molecule. Also provided are methods of binding epitope-bearing molecules (e.g., haptens) to the VP6 carrier molecule through binding peptides. The VP6 carrier can be a VP6 monomer, oligomer, or a particle comprisee of VP6 oligomers.

FIG. 1



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Description

THE USE OF ROTAVIRUS NUCLEOCAPSID PROTEIN VP6 IN VACCINE COMPOSITIONS

The present Inventi n relatas to immunological carriers and vaccine compositions. More particularly, the present invention relates to the us of rotavirus inner capsid protein VP6 as an immunologic carrier, as well as its use in e vaccine composition for use in stimulating immunity egainst rotavirus infectione.

Rotavirus is a genus of the family Reoviridee. This genus of viruses is widely recognized as the major caus of gastroenteritis of infants and young children in most areas of the world. In the lesser developed countrias diarrheal diseasas such as gastroenteritis constitute a major cause of mortality among infanta and young children. For a general background on rotaviruses, see Kapikian et al., in Virology, pp. 863-906 (B.N. Fields et al., eds., 1985), the disclosure of which is incorporated herein by reference.

Immunity to rotavirus infections and illness has been poorly understood. Animal studies, however, heve been conducted directed to the relative importance of systemic and local immunity. Bridger et al. (1981) Infect. Immun. 31:906-910; Lecce et al. (1982) J. Clin. Microbiol. 16:715-723; Little et al. (1982) Infect. Immun. 38:755-763. For example it has been observed that calves develop a diarrheal illness despite the presence of serum rotavirus antibody at the time of infection. Calves which are fed colostrum-containing rotavirus antibodies immediately before and after infection with rotavirus, however, do not develop diarrhea within the normal incubation period. See, e.g., Bridger et al. (1975) Br. Vet. J. 131:528-535; Woode et al. (1975) Vet. R c. 97:148-149. Similar results heve been achieved with newborn lambe, who developed resistance when fed colostrum or serum containing rotavirus antibodies for eeveral days during which period the lambs w re challenged with rotavirus. Snodgrass et al. (1976) Arch. Virol. 52:201-205.

In studies of the effect of edministering rotevirus to humans, it was found that a preexisting high titer of serum neutralizing antibodies to rotavirus correlated with resietance to diarrheal illness. Kapikian et al. (1983) Dev. Biol. Standard 53:209-218; Kapikian et al. (1983) J. Infect. Dis. 147:95-106. In infants and children, however, the presence of serum antibody to rotavirus has not been associated with resistance to infection or illness. See, e.g., Black et al. (1982) J. Infect. Dis. 145:483-489; Gurwith et al. (1981) J. Infect. Dis. 144:218-224; McLean et al. (1981) J. Clin. Microbiol. 13:22-29.

Most current efforts in experimental rotavirus immunoprophylaxis are aimed at the development of live attenuated virus vaccines. Attenuation, however, is usually essocieted with a decrease in tha level of viral replication in the target organ; i.e., the epithelium of the small intestine. Attenuated mutants of other mucosal viruses, however, have exhibited a diminished immune rasponse correlated with the decrease in replication. Since the protective efficacy of wild-type virus infection is marginal, it may be impossible to achieve the dasired immunoprophylaxis with a mutant exhibit decreased replication. Two bovine rotavirusas, NCDV and th UK strain, have been produced in attenuated form and evaluated as veccines in humans. Vesikari et al. (1983) Lancet 2:807-811; Vesikari et al. (1984) Lancet 1:977-981; Wyatt et al. (1984) in Conference Proceedings: Control and Eradication of Infectious Diseases in Latin America.

Another epproach to the development of an attenuated rotavirus vaccine is based on the ability of rotaviruses to undergo gene reassortment during coinfection. A number of "hybrid" strains have been isolat d from cultures coinfected with a wild-type animal rotavirus and a human rotavirus. Strains are selected which receive the gene coding for the outer nuclear capsid protein VP7, the remaining genes baing derived from the animal rotavirus parent. See, e.g., Immunogenicity, pp. 319-327 (Chanock & Lemer, eds., 1984).

Still another approach to immunization has been the suggestion of using recombinantly produced VP7 polypeptide in a vaccine. See, e.g., Virology, p. 892 (B.N. Fields et al., eds., 1985). It has been further suggested, however, that recombinant VP7 is unlikely to produce an effective primary local intestinal immune response. Id. at 893. The VP7 gene from several strains of rotavirus has been cloned and full-length or near fulllength cDNA has been attained. See, e.g., Arias et al. (1984) J. Virol. 50:657-661; Both et al. (1983) Proc. Natl. Aced. Sci. USA 80:3091-3095; Elleman et al. (1983) Nucleic Acid Res. 11:4689-4701; Floras et al. in Modern Approached to Vaccines; Molecular and Chemical Basis of Virus Virulence and Immunogenicity, pp. 159-164 (R.M. Chanock et al., eds., 1983).

It has also been suggested thet synthetic peptides corresponding to mejor anogenic sites of VP7 mey be useful in immunization. Virology, supra, p. 893. In addition, passive immunization with rotavirus antibodies has been shown to be effective in preventing rotavirue illness in animals and in infents and young children. Id.

The most abundent structural protain in rotavirus particlas ie the approximete 45 K MW nucleocapsid or inner capsid protein coded for by gene 6, known in the art as virus protein 6 or VP6. Although not an integral component of the outer capsid, it is an important viral antigen. It has been identified as the subgroup antigen by using several techniques including complement fixation, ELISA, immunoadherence agglutination assay, and specific monoclonal antibodies. VP6 is also described as the common rotavirus group antigen since some monoclonal antibodies against it will react with all rotaviruses, and polyclonal serum raised against a single rotavirus type can detect most other rotavirus strains. Aside from its antigenic properties, VP6 is very immunogenic and several investigators have found thet polyclonal serum raised to this protein has neutralizing ability. Bastardo et al. (1981) Infect. & Immun. 34:641-647.

Th gene encoding VP6 has been cloned. Se , e.g., Estes et al. (1984) Nucleic Acids R s. 12:1875-1887. VP6 has also been produced by recombinant m thods. Est s et al. (1987) J. Virol. 61:1488-1494.

Vaccine compositions for rotavirus disease comprised of peptides from VP7, VP6 and VP3 have also be n

propos d. Se commonly owned patent applications: U.S. Serial No. 903,325 (filed 3 September 1986); Australian Serial N . 526,116 (filed 23 December 1986); Australian Serial No. 66987/86 (filed 24 December 1986); Chinese Serial No. 86108975 (filed 25 December 1986); EPO Serial No. 86 117 981.0 (23 December 1986); and Japanes Serial No. 61-308945 (filed 26 December 1986), the disciosuree of which are incorporated by reference herein.

Several immun i gic carriers are known in the art, including, but not limited to, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin (OVA), beta-galactosidase (B-GAL), penicillinase, poly-DL-alanyl-poly-L-lysine, and poly-L-lysine. The coupling of the desired hapten or other epitope-bearing molecule to such carriere often requires elaborete chemical procedureé. Such procedures are expensive and mey heve e deleterious effect on the final complex comprised of the carrier and epitope-bearing molecule. Thus, there is a need in the art for improved immunological carriers to which epitope-bearing molecules can be attached readily, but which are also at least as effective as prior art immunologic carriers.

The present invention is based on the discovery that VP6 polypeptides of rotaviruses, or functional fragments thereof, in either monomeric or iigomeric forms, have the ability to bind peptides by virtue of an interscticn between the peptide and binding eite(s) on the VP6 polypeptide to form a VP6 - binding peptide complex. The present invention is also based on the discovery that VP6, in its monomeric or oligomeric forms, can be advantageously employed as an immunologic carrier to which moleculea bearing an epitope of interest can be attached. Preferably, these epitope-bearing molecules can be ettached to the VP6 polypeptide by use of a binding peptide. The above discoveries, therefore, provide for the production of compositione which can be used to stimulate an immune response to VP6, VP6 complex with an epitope- bearing molecule, as well as to the binding peptide if it is employed in the complex.

in one embodiment, the present invention is directed to a composition capable of raising an immunological responee in e memmal to a selected epitope comprising an immunological carrier complex, said complex comprised of an epitope-bearing molecule expressing said selected epitope, said epitope-bearing molecule being selected from the group consisting of polypeptides, carbohydrates and nucleic acide; said epitope-bearing molecule being coupled to a carrier protein selected from the group consisting of monom rs and oligomers of a polypeptide homologous to a rotavirus VP6 inner capsid protein amino acid sequence.

in several preferred embodiments of the above composition, the epitope-bearing molecule is a polypeptid , and the carrier protein is a VP6 inner capsid protein. in particularly preferred embodiments, the VP6 carrier protein is an oligomer formed into a particle, such as a tube or sphere. in a still further preferred embodiment, the epitope-bearing molecule is copied to the carrier protein through a protein-protein interaction with a binding peptide specific for the VP6 binding site(s).

in another embodiment of the present invention, an improved vaccine composition if provided wherein the epitope of interest is on a polypeptide bound to e carrier protein, the improvement comprising ueing rotevirue VP6 inner capsid polypeptide as said carrier protein.

in other embodiments of the present invention, veccination methods are provided, as well as specific binding peptidas.

Further embodiments of the present invention will readily occur to those of ordinary ekill in the art.

Figure 1 shows the nucieotide sequance of a cloned copy of the rotevirus strain S-A11 gene 6 encoding the polypeptide VP6. The sense strand (corresponding to the mRNA) is shown, as well as the predicted emino acid sequence of VP6. Termination sites are underlined. See Estes et al. (1984) Nucleic Acids R s. 12:1875-1887.

Figure 2 shows electron micrographs of particles produced from reassembled rotavirus VP6. Panel A shows particles from VP6 isolated from human strain WA rotevirus (subgroup 2), and panel B shows particles reassembled from recombinently produced VP6 from a baculovirus expression system.

Figure 3 is an electron micrograph of VP6 protein forming aggregated spherical particles in 0.01 M citrate buffer pH 4.0 and dialyzed to pH 5.0.

Figure 4 is an electron micrograph of VP6 protein reassembled into various forms by dialyzing first to 0.01 M phosphate buffer, pH 6.0, and then to 0.01 M citrate buffer, pH 4.0, at 4°C. The micrograph shows hexamers, small hexagonal lattices and tubes as well as sheets (arrows) consieting of a small-hole lattice. The arrow on the figure indicates the corresponding sheet on the originel micrograph. Bars represent 100 nm.

Figure 5 ie a echematic representation of the assembly of VP6 monomer into various oligomeric structures.

Figure 6 depicts dose-response curves to spherical VP6 cerrier protein with and without various epitope-bearing moleculas complexed therewith.

Figure 7 depicts dose-response curves to spherical VP6 carriers complexed with or without various epitope-bearing molecules.

Figure 8 depicts dose-response curves to spherical VP6 carrier protein with or without epitope-bearing molecules complexed therewith.

Figure 9 depicts a dose-r sponse curve for a spherical VP6 carrier protein complexed with an epitope-bearing molecule.

in describing the present invention, the following terms will b employed, and are intended to be the defined as indicated below. .

An "immunological response" to an epitope of interest is the development in a mammal of either a cell-or

antibody-medieted immun respons t the pitope of interest. Usually, such e respons consists of the mammal producing antibodies and/ r cytotoxic T cells direct d specifically to th epit pe of int rest.

An "immunological carrier complex" refers to e chemical complex betwe n a immunologic carrier m lecule, usually a prot in, and e hapten or other pitope-bearing mol cule. Th epitope on the hapten or other epitope-bearing molecule for which an immunological response is desired is referred to as th "epitop of interest" or the "selected epitop ".

An "epit pe-bearing molecule" refers to e molecule within an immunological carrier complex which is bound to the carrier molecule and bears the epitope of interest. The epitope-bearing molecule of the pres nt invantion can include, but is not limited to, polypeptides, carbohydretes, nucleic acids, and lipids. Further examples are given below.

A "rotavirus VP6 inner capsid protein" refers to the art-recognized mejor viral protein of the inner capsid from any species or strain within the genus Rotavirus. See, e.g., Kapikian et al., supra. Examples of rotavirue strains from which the VP6 protein can be isoleted and employed in the present invention include, but are not limited to, Simian SA-11, human D rotavirus, bovine UK rotavirus, human We or W rotavirus, human DS-1 rotavirus, rhesus rotavirus, the "O" agent, bovine NCDV rotavirus, human K8 rotavirus, human KU rotavirus, human DB rotavirus, human S2 rotavirus, human KUN rotavirue, human 390 rotavirus, human P rotavirus, human M rotavirus, human Walk 57/14 rotavirus, human Mo rotavirus, human Ito rotavirus, human Nemot rotavirus, human YO rotavirus, human McM2 rotavirus, rhesus monkey MMU18006 rotavirus, canin CU-1 rotavirus, feline Taka rotavirus, equine H-2 rotavirus, human St. Thomas No. 3 and No. 4 rotaviruses, human Hosokawa rotavirus, human Hochi rotavirus, porcine SB-2 rotavirus, porcine Gottfried rotavirus, porcine SB-1A rotevirue, porcine OSU rotavirus, equine H-1 rotavirue, chicken Ch.2 rotavirus, turkey Ty.1 rotavirus, and bovine C486 rotavirus. Thus, the present invantion encompasses the use of VP6 from any rotavirue strain, whether from subgroup I, subgroup II, or any as yet unidentified subgroup, as well es from any of the serotypes 1-7, as well as any as yet unidentified serotypas. Furthermore, the present invention encompesses the use es an immunologic carrier of polypeptides having homologous amino acid sequences to rotavirus VP6 amino acid sequences which are unique to the class, or any member of the class, of VP6 polypeptides. Such unique sequences of VP6 proteine are referred to as e "rotavirus VP6 inner capsid protein amino acid sequence".

"Oligomers" refer to multimeric forms of, for example, VP6 polypeptides. Usually, such VP6 oligomers are trimers formed by intermolecular disulfide bridging between VP6 monomers. See, e.g., Figure 5.

The binding of an epitope-bearing molecule to e VP6 carrier protein through "protein-protein interection(e)" refers to the type of chemical binding, both covalent and non-covalent, between e binding peptide region of the epitope-binding molecule and the VP6 carrier molecule. The exact nature of this binding is not understo d. It is characterized, however, es the binding phenomenon observed when e peptide, heving e Cys and another charged amino acid (e.g., Arg) in a structural reletionship to each other analogous to thet shown in peptide A or B (below), binds to VP6 binding sites on the carrier molecule through mere mixing of VP6 carrier protein and molecules containing the binding peptide region. It is believed that this protein-protein interection is e combination of e disulfide bridge involving the Cys, and e non-covalent interaction involving the changed amino acid, but applicants do not wish to be bound by this theory.

A "binding peptide" refers to amino acid sequences which heve the ebility to bind through a protein-protein interaction with e VP6 polypeptide. Thase binding peptides are discussed in more detail below.

A composition "free of rotavirus virions" refers to e composition which does not contain intact virus particles, although it mey contain particles formed from VP6 complexed to other molecules.

A "vaccine composition", according to the present invention, is an otherwise conventional vaccine formulation employing either VP6 polypeptides alone or in an immunological carrier complex es the active ingredient. The preparetion of vaccines containing the above ective ingredients is well understood int he art. Typically, vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection mey also be prepared. The preparetion may als be emulsified or the ective ingredient encapsuleted in liposomes. The ective immunogenic ingredient is often mixed with excipients which are pharmeceutically acceptable end compatible with the ective ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinetions thereof. In eddition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering egents, or adjuvants which enhance the effectivenees of the vaccine. The vaccines are conventionally adminstered parenterally, by injection, for example, either subcutaneously or intramuscularly. Injectable vaccine formuletions will contain an effective amount of the ective ingredient, the exact amount being readily determined by one skilled in the art. The ective ingredient can range from about 1% to about 95% (w/w) of the injectable composition, or even higher or lower if appropriete.

Additional vaccine formulations which are suitable for other modes of edministration include suppositories and, in some cases, oral formuletion. For suppositories, the vaccine composition will include traditional binders and carriers, such as, polyalkaline glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the renge of about 0.5% to about 10% (w/w), preferably about 1% to about 2%. Oral formulations include such normally employed xcipients as, for exampl , pharmaceutical grades of mannitol, lactose, starch, magnesium, stearate, sodium saccharin cellulose, magnesium carbonete, and the like. These oral vaccine compositions mey be taken in the form of solutions, suspensions, tablets, pills, capsul s, sustained release formuletions, or powders, and contain from about 10% to about 95% of the active ingredi nt, preferably about 25% to about 70%.

Furthermore, th VP6 proteins or immunological carrier compl xee of the present invention mey b formuleted into vaccine compositions in either neutral or salt forme. Pharmeceutically acceptabl salts include the ecid eddition salts (formed with the free amino groups of the ective polypeptides) and which are formed with inorganic ecids such as, for example, hydrochloric r phoephoric ecids, r such organic acids es acetic, oxalic, tartaric, mandelic, and the like. Salts formed from free carboxyl groups mey also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and euch organic basee es isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The vaccine composition of the present invention may be edministered in e manner competible with th dosage formulation, and in such amounts as will be therapeutically effective and immunogenic. The quantity t be edministered depends on the subject to be treeted, the capecity of the subjects immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of ective ingredient desired to be edministered depend on the judgment of the prectioner and are peculiar to eech eubject. The estabilehment of effective dosages for e particular formuletion, however, are within the ekill of the art through routine triels establishing dose-response curves.

The rotavirus genome consists of eleven segments of double-stranded RNA. These 11 genes encode for the production of et least six structural proteins of the virus. In complete virus particles, these six proteins occur in a double-shelled arrangement. There are three inner shell (capsid) proteins designeted virue protein (VP) 1, 2, and 6. There are three outer capsid proteins, two of which are designeted VP3 and VP7. The third outer capsid protein, which is encoded by genomic segment 10 or 11, has not yet been assigned e number. The molecular weights of these proteins are shown in Table 1.

Table 1
Gene assignment and Molecular Weight
of the Major Rotavirus Structural Proteins

Genomic Segment	Protein Designation	Molecular Weight	Location*
1	VP1	110K	inner
2	VP2	92K	inner
4	VP3	84K	outer
6	VP6	45K	inner
7			
8 triplet	VP7	41K	outer
9			
10 or 11	ND	20K	outer

* Designates location of the structural protein in the inner or outer capsid of complete rotavirus particles.

In different roteviruses, the ebsolute order of the genomic segments does not elways correspond to the same genes. For example the electrophoretic order of segments 7, 8, and 9 changes among roteviruses from different animal species. This is referred to as inversion or "flip-flopping" of genome segments. The gene triplet formed by segments 7, 8, and 9 codes for three polypeptides, the neutralization-specific mejor outer capsid glycoprotein identified es virus protein (VP) 7 and two nonstructural proteins which are now shown in the table. In rotavirus strains SA-11, W, and Wa, gene 9 codes for VP7. In rotevirus strain DS-1 and UK bovine rotevirus, however, gene 8 codes for VP7. There are discrepancies in the literature about the exact molecular weight of VP7, as well as of other rotevirus proteins. Severel researchers heve suggeeted thet this is in part due to the many varietions in methods used to: (1) separate the individual polypeptides, (2) prepare virus samples for electrophoresis, (3) detect polypeptides in polyacrylamide gels, and (4) detect various post-translational modifications of primary gene producte. In addition, especially for bovine and human rotavirus, there are varietions in the mobility of proteins derived from different isoletes orignieting from the seme species. The molecular weights shown in Table 1 are those reported by Sabara et al. (1985) J. Virol. 53:58-66.

As discussed ebove, VP6 is the most abundant of the inner capsid proteins, constituting about 80% by w ight of the inner shell. Roteviruses can be divided into two subgroups (I or II) based on an pitope on VP6 which can be identified using monocional antibodies. Most roteviruses examined to dete fall into one of the two subgroups; however, there is evidence that both subgroup epitopes can b located on e single VP6

molecules. For example, recently an equine rotavirus was identified as having both subgroup 1 and 2 epitopes on VP6. See, e.g., Hoshino et al. (1987) Virology 157:488-496. Therefore, it is not inconceivable that the subgrouping classification may be extended or modified as new isolates are identified and their genes sequenced. There are also at least 7 serology groups into which rotaviruses have been classified.

All VP6 molecules sequenced to date consist of 397 amino acids, although some variability in the molecular weight of the protein has been reported which may indicate a protein with more or less than this number of amino acids. Specifically, the raported molecular weight range for VP6 is 41-45K, thereby indicating an amino acid size range of 397-425. However, molecular weight variability does not necessarily reflect a difference in the number of amino acids but can be due to electrophoratic conditions used in characterization of the protein. Only by sequencing the gene coding for a particular VP6 can the number of amino acids be determined (See, e.g., Figure 1). The amino acid homology between VP6s belonging to the two different subgroups is 80% or more, based on the VP6 genes sequenced to date.

Within rotavirus, monomeric units of VP6 exist in a variety of oligomeric forms. Trimeric units (molecular weight about 135K) occur in both the virus particle and in infected cells, with the intersubunit linkage consisting of non-covalent interactions. These trimeric units complex further by virtue of disulfide bridges into larger units which likely represent the ring-like structuree observed using electron microscopy. By employing different sample buffers, these nucleocapsid oligomeric complexes can be visualized on polyacrylamide gels.

VP6 protein can be prepared by any of several methods. First, VP6 can be purified from in vitro-derived single-shelled virus particles by calcium chloride ($CaCl_2$) or lithium chloride (LiCl) treatment by standard techniques. See, e.g., Almeida et al. (1979) J. Med. Virol. 4:269-277; Bican et al. (1982) J. Virol. 43:1113-1117; Gorziglia et al. (1985) J. Gen. Virol. 66:1889-1900; Ready et al. (1987) Virology 157:189-198. Alternatively, VP6 can be produced by recombinant DNA techniques, which are fully explained in the literature. See, e.g., Manietis, Fritsch & Sambrook, Molecular Cloning: A Laboratory Manual (1982); DNA Cloning, Volumes I and II (D.M. Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1985); Transcription and Translation (B.D. Hames & S.J. Higgins eds. 1984); Animal Cell Culture (R.I. Freshney ed. 1986); Immobilized Cells and Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984).

DNA coding sequences encoding VP6 polypeptides can be derived from VP6 mRNA. See, e.g., Estes et al., supra; Both et al. (1984) J. Virol. 51:97-101; Cohen et al. (1984) Virology 138:178-182. Alternatively, a DNA sequence encoding VP6 can be prepared synthetically rather than cloned. The DNA sequence can be designed with the appropriate codons for a VP6 amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge (1981) Nature 292:756; Nambair et al. (1984) Science 223:1299; Jay et al. (1984) J. Biol. Chem. 259:6311.

Once a coding sequence for VP6 has been prepared or isolated, it can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Example of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage lambda (E. coli), pBR322 (E. coli), pACYC177 (E. coli), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-E. coli gram-negative bacteria), pHV14 (E. coli and Bacillus subtilis), pBD9 (Bacillus), pHV14 (E. coli and Bacillus subtilis), pBD9 (Bacillus), pIJ61 (Streptomyces), pUC6 (Streptomyces), YIp5 (Saccharomyces), YCp19 (Saccharomyces) and bovine papilloma virus (mammalian cells). See generally, DNA Cloning: Vols. I & II, supra; T. Maniatis et al., supra; B. Perbal, supra.

The coding sequence for VP6 can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding VP6 is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. In bacteria, for example, VP6 is preferably made by the expression of a coding sequence containing a leader sequence which is removed by the bacterial host in post-translational processing. See, e.g., U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397.

An expression vector is constructed so that the VP6 coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). The control sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

A number of procaryotic expression vectors are known in the art. See, e.g., U.S. Patent Nos. 4,440,859; 4,436,815; 4,431,740; 4,431,739; 4,428,941; 4,425,437; 4,418,149; 4,411,994; 4,366,246; 4,342,832; see also U.K. Patent Applications GB 2,121,054; GB 2,008,123; GB 2,007,675; and European Patent Application 103,395. Yeast expression vectors are also known in the art. See, e.g., U.S. Patent Nos. 4,446,235; 4,443,539; 4,430,428; see also European Patent Applications 103,409; 100,561; 96,491.

Depending on the expression system and host selected, VP6 is produced by growing host cells transformed

by an expression vector described above under conditions whereby the VP6 protein is expressed. The VP6 prot in is then isolet d from the host cells and purified. If the expression system secret s the VP6 int growth media, the protein can be purified directly from cell-free media. If the VP6 protein le not secreted, it is isolsted from c il lysat s. Th eelection of th appropriate growth conditions and recovery methods are within the skill of the art.

Purified VP6 protein exhibits structural polymorphism. Specifically m hexamers and small hexegonal isttices are present in many of the samples. Tubular particlas form between about pH 5.0 and about pH 9.0, and are moderately stable t changes in temp reture and ionic strength. Th formation of thes particlas is fully reversible. Spherical particlee reassembling single-shelled virus can be formed et about pH 4.0. A novel etructure, in the form of sheete, composed of emall-hole isttice, is formed in samplee shifted from about pH 6.0 to about pH 4.0. These resuits demonstrate the importance of VP6 and of protein-protein interactions for rotevirus assembly.

Such protein-protein interactions are likely involved in the observed phenomenon thet certain peptides can bind to VP6 in its monomeric form or to various oligomeric structures formed from VP6 monomers, such as in vitro assembled tubes and spheres. The ettachment is medieted by s epecific binding site(s) within VP6. The structures which rasult from this binding, i.e., VP6 with s bound peptide, shall be referred to as VP6 binding peptide complexes. They can function as carriers to which other molecules bearing an epitope of interest (e.g., haptens) can be sttached. By definition, therefore, VP6 bound to another molecule by virtue of s specific amino ecid sequence (binding peptide), which occure neturally or has been tailored onto the epitope-bearing molecule, can be defined as an immunologic carrier for such s molecule.

Many molecules are known in the art thst bear an epitope and which can be useful when ettached to e carrier. Examples of the classes of such molecules, ususlly mscromolecules, are polypeptides, carbohydrates, and nucleic ecids. Proteins, glycoproteine, and peptides can include cytokines, hormonas, glucagón, insulin-like growth factors, growth hormone, thyroid stimulating hormone, prolectin, inhibin, secretin, neurotensin, cholecystokinin or fragments thereof, calcitonin, somstostatin, thymic hormones, neurotrensmittere and blockers, peptide-releasing factors (e.g., enkephalins), growth hormone releasing factor, as well as antigenic fragments of proteins, such as calmodulin, E. coli heet stable and heat labile enterotoxin, cholera toxin; and enzymes, such as protein kinase of Rouse sarcome virus. Additional polypeptides include steroid hormones, such as testosterone, estradiol, aldosterone, endrostenedione, or fragments thereof. Examplee of nucleotides include polynucleotide fragments, restrictions enzyme sites, and cyclic nucleotides (e.g., cyclic edenosine monophosphete). Examples of carbohydrates and cerbohydrete complexes include bacterial cepsules or exopolysaccharides (e.g., from Hemophilus influenzae B), bacterial lipid A essocieted core antigens (e.g., from Pseudomonas species), blood group antigens (e.g., the ABO antigens), and glycolipids. Examples of lipids include fatty acids, glycerol derivatives, prosteglandins (e.g., prostaglandin E_2), and lipopeptides (e.g., leukotelene B_4). Molecules of interest can aleo include alkaloids, such as vindolin , serpentine, catharanthine, as well as vitamins containing -OH, NH, SH. CHO, or COOH functional groups.

In order to sttach molecules to VP6 carriers, one mey employ conventional chemical coupling techniques. A particular sdvantege of the VP6-binding peptide complex as e carrier, however, is thst thle system facilitates the ettachment of molecules with minimal manipuletion. For example, s synthetic peptide corresponding to an antigenic or immunogenic region of s particular infectious egent (the epitope of interest) can be chemically synthesized in such s way thst it also contains the amino ecid sequence (binding peptide) necessary to link it to VP6. This can be done without altering the antigenicity of the region to which immune responses are sought and mey enhance the immunogenicity of this region. The antigenic region can also be produc d vie recombinant DNA technology, as describe above, in which case the nucleotide sequence coresponding to the binding peptide can be edded so thst the resulting product is s combinetion (fusion protein) of the antigenic region and the binding peptide. Attachment of the molecule to the VP6 carrier is then simply achieved by mixing the two substances without additional manipuistion.

Several peptides hsve been found or dasigned thst bind to VP6. The smino scid sequences for two are:

(1) Peptide A (22 amino acids): Cys-Asp-Gly-Lys-Tyr-Phe-Ale-Tyr-Lys-Val-Glu-Thr-Ile-Leu-Lys-Arg-Phe-His-Ser-Met-Tyr-Gly, and

(2) Peptide B (25 amino scids): Cys-Asn-Ile-Ais-Pro-Ale-Ser-Ile-Val-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ala-Gln-Pro-Asn-Gln-Asp-Ile-Ala.

Both peptides A and B occur neturally es portions of virus protein 3 (VP3) of roteviruses and are sensitive to trypsin. Clesvage of the peptides by trypsin prevents them from binding to VP6. It is clesr thet both of the sequences which are given herein ere by way of exemple only, and thet other compositions releted to binding sequences, or sequences in which limited conservetive amino acid changes are introduced, can also be used. Indeed, as described below, additional binding peptides can be designed by those of ekill in the art in light of the present disclosure. For exsmple, variant peptides derived from peptide B were further investigated in order to delineete the feetures of the peptide which are importent for binding to VP6. The features relste to the spstial arrangement of a cysteine and arginine residue, and the three-dimensional conformstion of s peptide which allows it to bind to VP6. Therefore, any peptide which exhibits these characteristics can be considered as a binding peptide.

Below are examples of specific embodiments for carrying out the present invention. The examplas are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Examples

1. Production of VP6

A. Isolation of Native VP6

Bovina rotavirus isolat C486 was propageted and purifiad as previously described. Sabara t al. (1986) J. Gan. Virol. 68:123-133. Briefly, virus was grown in confluant African monkey kidney cells (MA-104) in th absence of fetal bovine serum and in tha presence of 10 ug trypsin/ml. Virus was purified by differential cantrifugation and pallatad for 2 hours et 100,000 xg through a 40% sucrose cushion. Aftar resuspension in watar, virus was stored et -70°.

Nuclaocapsid protain was isolatad by succassiva dagradation of purified virus with EDTA and aither $CaCl_2$ or LiCl, as follows. Outar capsid proteins wara removad by incubeting virus (3 mg/ml) in 50 mM EDTA - 0.01 M Tris-HCl pH 7.4 at 4° for 30 minutes. Subviral particles were recovered by ultracentrifugetion (100,000 xg, 2-3 hrs, 4°) and resuspended in 0.01 M Tris-HCl pH 7.4 or 0.01 M sodium borate pH 9.0. They were than treated with either 1.5 M $CaCl_2$ - 0.01 M Tris-HCl pH 7.4 at 20° for 20-30 minutes or frozan in 2 M LiCl - 0.01 M sodium borata pH 9.0 at -70$_0$ for 4 days. Cores and undagraded particlea ware separated from solubilizad protain by ultracantrifugation. EDTA and salts wara removed by extansive dialysis et 4° egainst 0.01 M Tris-HCl pH 7.4, unless otherwisa indicated. Tha purity of the samples was axaminad by polyacrylamida gal alactrophoresis (PAGE) Laemmli (1970) Nature 227:680-685.

B. Recombinant VP6

To produca tha racombinant VP6, gena 6 of bovine rotavirus C486 was first clonad in tha Pst1 site of pBR322. The resulting clona was digestad with AhaIII and HpaII and subcloned into tha Sme I sita of pAC373. After transfection into Escherichie coli, plasmids in recombinant ampicillin resistant colonies were scraaned by restriction enzyma analysis for inserts in the correct transcriptional orientation. To transfer gana 6 cDNA from the pAC373 vector to tha Autogrepha califomica nuclear polyhadrosis virus (AcNPV) DNA, Spodopt re frugiperda cells were cotransfected with wild-type AcNPV DNA using tha calcium phosphete precipitation procedure as previously described. Smith et al. (1983) J. Virol. 46:584-593. Following incubation et 27° C for 4 hrs, tha medium was removad and tha cells observad with an inverted microscopa for signs of infection. The extracellular virus was harvested et 5 deys post-infection and plaqued on Spodoptere frugiperde cell monolaysrs. Recombinants wera salectad by idantifying occlusion negativa plequas with an inverted microscopa. Positive pleques were further grown in microtiter dishas and nucleic acid dot blots on infected cells in theee dishes were performed to verify the presence of gana 6. Plequa purification of positive supemetants from microtiter wells was performed and the virus from thase plaques was used to propagat virus stocks.

To isoleta VP6 from infected cells, the cells were first lysed with a buffer containing 1% NP40, 0.137 M NeCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 0.1 mg/ml aprotinin. The lysate was then dialyzed in .01 M citrate buffer pH 4.0 for 48 hrs during which time e precipitate which represented reassambled VP6 formad in the dialysis beg. The pracipitata was then collected by centrifugetion, then treetad with 0.05 M EDTA pH 5.0 for 1 hour and recentrifuged. The resulting pellet contained purifiad VP6 reassembled epheres.

Rotavirus C486 is publicly available from the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville, MD 20852, USA, where it was dapositad under Accassion No. VR-917 on 15 April, 1981. The pAC373 vactor containing the rotavirus gena 6 cDNA was designeted pAC373BRV6 and deposited with tha ATCC on 31 August 1987 undar Accession No. 40362, where it will be maintained under the tarms of tha Budap st Treaty.

2. Binding Paptides

Savan different synthetic peptides were tested for the ability to bind VP6. Tha primary structure of tha peptidas was as follows:

Paptida A C-D-G-K-Y-F-A-Y-K-V-E-T-I-L-K-R-F-H-S-M-Y-G
Peptide B C-N-I-A-P-A-S-I-V-S-R-N-I-V-Y-T-R-A-Q-P-N-Q-D-I-A
Peptide C Y-Q-Q-T-D-E-A-N-K
Peptida D D-E-A-N-K-K-L-G-P-R-E-N-V-A
Peptida E R-N-C-K-K-L-G-P-R-E-N-V-A
Peptide F R-N-C-K-K-L-G-P-R-M-M-R-I-N-W-K-K-W-W-Q-V
Peptide G T-N-G-N-E-F-Q-T-G-G-I-G-N-L-P-I-R-N-W-N

Tha various peptides ware reacted for 30 minutas at 37°C with 2.0 ug of purifiad VP6 from bovina rotavirus strain C486. Binding was then tested by gel electrophoresis. Two of these synthetic peptides (peptides A and B) bound to VP6 protein in the gel. A "leddering" effect was seen et locations corresponding t the 45K (molacular weight of VP6 monomar), 90K (molecular weight of VP6 dimer) and 135K (molacular waight of VP6 trimer) regions. Additional support for the binding of tha two peptides to the various forms of VP6 was provided by tha fact that the molecular weight increments in aach ledder correspondad to tha molecular waights of tha synthetic peptide monomere. Definitive proof that the paptid bound t tha VP6 prot in was damonstrated by the fact that a ladder was d tected at both the 45 K and 90K regions with antisera produced

against the synthetic peptides.

In order t further delineate the feetures of the binding peptide required for binding to VP6, sev ral variant peptides deriv d from peptide B (also referred to as 84 TS) were synthesiz d and tested for their ability to bind to VP6. A list of the variant peptidee along with their amino acid sequence and their binding ability is shown in Table 2, below.

Table 2
VARIANT PEPTIDES DERIVED FROM PEPTIDE B (84TS)

NAME OF PEPTIDE VARIANTS	AMINO ACID SEQUENCE[1] 1 5 10 15 20 25	BINDING TO VP
84 TS (PEPTIDE B)	C N I A P A S I V S R N I V Y T R A Q P N Q D I A	+
84 TS-CYS	* C	-
DISER	* * * * * * * * * S * * * * * * S * * * * * * * * *	-
MONOSER	* * * * * * * * * * * * * * * * * S * * * * * * * * *	-
SHT	C G A * * * * * * * * * * * * *	+
SR-SHT	C G A * S * * * * * * * * * * *	
CP-41-SHT	D T F E G A P A C G A * * * * * * * * * * * * *	+

[1]Amino acids are numbered 1-25 starting at the amino terminal end of Peptide B.
[2]The asterisks (*) indicate conserved amino acids from Peptide B.

The importance of the cysteine residue located on the binding peptide with respect t VP6 binding was apparent due to the fact that th reducing egent B-mercaptoethanol was abl t abolish binding as discuss d

below in Example 4. However the presence of a cysteine residue is not the only requirement for binding to VP6 as illustrated by the fact that the 84 TS-Cys peptide, which has the cysteine residue at its carboxy terminal instead of the amino terminal end, does not bind VP6. It was therefore hypothesized that the position of the cysteine relative to another charged residue, having the ability to interact electrostatically with charged residues on VP6, was also important. The other predominant charged residues on the parent peptide B are 2 arginines at positions 11 and 17. In order to test whether the arginine residues were indeed the important charged residues, 2 variant peptides were made. Specifically, the monoser variant peptide had arginine 17 replaced by an uncharged amino acid (serine) and the diser variant peptide had both arginine 11 and 17 replaced by serines. Since neither the monoser or diser bound to VP6, it appears that at least arginine 17 or both arginine 11 and 17 are required for binding to VP6.

The importance of the cysteine and arginines was further illustrated by the fact that a portion of peptides B (84 TS) could be deleted to produce the SHT peptide and still maintain binding to VP6. Specifically, amino acids 1-09 and 19-25 of peptide B were deleted and 3 amino acids including a cysteine were added to the amino terminal end, thereby decreasing the size of peptide B by 50%. Even though a cysteine residue is one of the requirements for peptide binding, its position appears to be somewhat important relative to that of the charged residues. For example, the peptide gp-41-SHT has a cysteine located in position 7 relative to the numbering system for peptide B, but its distance from the arginine residue is similar to that in peptide B and consequently binding to VP6 is observed.

In summary, the features important for peptide binding to VP6 relate to the spatial arrangement of a cysteine and arginine (or the charged amino acid) residues in the tree-dimensional conformation of a peptide. Any peptide which has these features and consequently can bind to VP6 can be considered a binding peptide. An example of such a peptide is peptide A, which is derived from a sequence on the rotavirus VP3 protein, and is only related to peptide B in that it has a cysteine and arginine residue in the proper arrangement to allow binding to VP6.

3. VP6 Derived from Various Sources for Use as a Particle Carrier With or Without the Binding Peptid

Preliminary studies into the ability of VP6 to reassemble and to bind peptides in Example 2 were carried out using VP6 derived from bovine rotavirus strain C486. This virus strain belongs to subgroup I, and the epitope determining subgroup specificity is located on VP6. In order to determine whether VP6 derived from other sources will exhibit the same two properties (i.e., reassembly and binding of peptides), SP6 derived from a subgroup II human rotavirus strain (strain WA) and a subgroup I VP6 produced by recombinant DNA technology (Example 1) were tested. The importance of testing a recombinant DNA product is that protein processing may not be the same as that in a natural infection, even though the genetic information is identical. If the processing is different, the resulting protein product may not have the intrinsic features necessary for reassembling or peptide binding. The recombinant DNA VP6 was produced as described in Example 1.

The testing for the ability of VP6 to reassemble was carried out as follows. First, preparations containing no less than 0.1 ug of VP6/ul isolated from the subgroup II rotavirus or recombinant DNA-produced VP6 were dialyzed at 4°C against 1 liter of 0.01 M citrate buffer at pH 4.0 for 36 hours, with three changes of buffer during this time interval. Second, after dialysis, an aliquot of the preparation was examined by electron microscopy for the presence of particles. Figure 2 illustrates that both subgroup II VP6 (Panel A) and recombinant DNA-derived VP6 (Panel B) can reassemble in spherical and tubular particles, indicating that they have the intrinsic features necessary for this type of process to occur.

The ability of the various VP6s to bind peptide was also tested. Preparations containing subgroup II rotavirus or recombinant DNA-produced VP6 were mixed with peptide B in a ratio of 1:10 (w/w). The mixture was then electrophoresed on a 10% polyacrylamide gel. Both subgroup II VP6 and recombinant DNA-derived VP6 were able to bind peptide as illustrated by a laddering in the region of the gel containing VP6.

Therefore, it appears that the features necessary for VP6 reassembly and peptide binding are present on both VP6 subgroups, various mammalian rotavirus VP6s, and recombinant VP6.

4. Characterization of VP6-Monomer-Binding Peptide Complex

Further characterization of the conditions required for binding of peptides in VP6 was carried out using peptide B.

Two micrograms of radiolabeled double-shelled rotavirus was reacted with 100 ug synthetic peptide B for 30 min, 37°C. Prior to electrophoresis, the sample was aliquoted and treated with one of several buffers. The VP6-peptide B complex was treated with Laemmli buffer (0.0625 M Tris-HCl pH 6.6, 4% sodium dodecyl sulfate, 8% glycerol and 0.05% bromphenol blue) for 30 min at 37°C. The same laddering effect described in Example 2 was observed. However, when B-mercaptoethanol was included in the sample buffer and the sample was boiled prior to electrophoresis, the ladders in both the 45K and 90K regions disappeared. This suggested that disulfide bridging was necessary to maintain the VP6-peptide B complex. However, the interaction between VP6 and peptide B could withstand such harsh treatments as boiling in sodium dodecyl sulfate. Identical results were obtained with subgroup II VP6, recombinant DNA-derived VP6 and the other binding peptides.

5. Characterization of VP6-Assembled Particles-Binding Peptid Complex

Binding of the peptide B to in vitro-assembled tubular and spherical particles c mposed of th VP6 monomers was also observed. These in vitro-assembled particles were produced by subj cting isolated VP6 to different pH c nditions. Specifically, when isolated VP6 was placed in 0.01 M citrate buffer pH 4.0 and dialyzed to pH 5.0, high aggregated, mpty spherical particles occurred (Figure 3). Tubular particles formed at pH 5.0 t 9.0 and aggregated at pH 5.0 (Figure 4). VP6 was dialyzed first t 0.01 M phosphet buffer (pH 5.0) and then t 0.01 M citrate buffer (pH 4.0) at 4°C. Th surface structure of th particles appears well ordered at pH 5.0 to 7.0 and less well ordered et pH 8.0 and 9.0. In addition to hexamers, small hexagonal lattices and tubes, the sample contained sheets (arrows) consisting of a small hole lattice. At the higher pH levels there was more amorphous material present than et pH 5.0 to 7.0, suggesting that perhaps less of the protein had polymerized. Figure 5 summaries the relationship between VP6 monomers and VP6 oligomeric structures.

The spherical particles hed e diameter of 62.0 ± 15 nm (n = 56) which le consistent with that f single-shelled virus particles. Tubes were 104 ± 15 nm (n = 56) in diameter. Immunoelectron microscopy and immunogold labelling were used to show that the tubes consisted of the VP6 protein. Specifically tubes were labeled with immunogold when monoclonal antibody specific for VP6 was used as the primary antibody, but were not labeled when normal mouse serum was used as the primary antibody.

To confirm that the synthetic peptides could bind to these in vitro-assembled particles, gride were coated with antiserum to VP6, or with antiserum against peptide B. The number of tubular particles trapped on equivalent areas of the two types of grid was then counted. When tubular particles were not reacted with peptide B, grids coated with antibodies to VP6 trapped over 30 times as many tubes as did grids coated with antibodies against peptide B. However, when tubular particles were first reacted with peptide B, then th number of tubular particles trapped on gride coated with antibodies against the peptide B was at least 5 times as large as for unreacted tubular particles (Table 3).

Table 3

IMMMUNOSORBENT SERUM ELECTRON MICROSOPY OF VP6 TUBES WITH AND WITHOUT PEPTIDE B

Antibody Used to Coat Grid	Sample	Number of Tubes/ Counted Area
Antiserum to VP6	Tubes	300
Antiserum to Peptide B	Tubes	10
Antiserum to Peptide B	Tubes with Peptide B	53

The binding of the peptide B to in vitro-assembled spherical and tubular structures was further confirmed by the observation of a ladder formation of the VP6 protein derived from these particles on e polyacrylamide gel.

The specific nature of this binding phenomenon was investigated further by examining the primary amino acid sequence of the peptide binding site, and the number of VP6 binding sites. The conditions for binding to occur have already been described above.

The number of potential binding sites on VP6 can be estimated by conting the number of rungs on a VP6 ladder formation. There is e shift from one rung to four rungs as the ratio of peptide to VP6 increases from 2:1 to 25:1. This indicates thet there may be as many as four VP6 binding sites. However, since the synth tic peptide forms dimers of itself in solution, it is not possible to determine, via this type of experiment, whether there are four primary VP6 binding sites or two primary VP6 binding site, with bound peptide dimers t each VP6 binding site.

6. Immunogenicity of the Various Forms of VP6

The immunogenicity of the VP6 monomer and of tubular and spherical forms of VP6 assembled into particles (Figure 4) were investigated. As illustrated in Table 4 below, both particle types were very immunogenic based on e comparison of antibody titers produced after immunizing mic with 10 ug of either th VP6 monomer, spherical particles, tubular particles or naturally occurring incomplet virus particles. Th immunogen was administered three times over an ight-week period and was emulsifi d in Freund's incompl t Adjuvant.

Table 4

Immunogenicity of Various Forms of VP6 Monomeric and Oligomeric Structures as Compared to Incomplete Rotavirus Particles

Form of VP6 Used for Immunization of Mice -	Antibody Titer Determined by Enzyme-linked Immunosorbent Assay Using the Incomplete Virus Particle as the Capture Antigen
VP6 Monomer	$10^{4.5}$
Tubular Structure	$10^{6.5}$
Spherical Structure	$10^{7.9}$
Incomplete Virus	$10^{7.0}$

7. Examples of Immunizing with VP6 Assembled Particles -Epitope Constructs

This Example demonstrates the sfficacy of ths VP6-assembled particles as an immunological carrier for epitopes whose amino acid sequences were derived from parasitic, becterial and viral immunogens. Thes represent protein and glycoprotein haptens as well as a bacterial carbohydrete molety which demonetretee the utility of the carrier with heptens other than those of protein origin.

A. Production of VP6-Assembled Particlas (spherical carrier)

Bovine rotavirus (strain C486 rotevirus subgroup I was grown in MA-104 cells (monkey kidney), harvested, then purified and concentreted by uitracentrifugation. The VP6 was extracted from purified virus preparations by successive treatment with ethylene-diamine tetre acetic acid (EDTA) and lithium chloride ($LiCl_2$). Preparations containing VP6 were then dialyzed to pH 4.0 et which time a precipitate formed, repres nting aggregated spherical particles, as described above. The aggregated spheres were dispersed by dialysis t pH 5.0 or higher and then were etored et -70° C.

Verification of the composition of the peticles was by gel electrophoresis and immunoblot ELISA, using antisera specific for VP6. Verification of the ultrastructure of the particles was by electron microscopy.

B. Synthesis of SHT Peptide-Epitope (Hapten) Constructs

SHT peptide-epitope (hapten) constructs wsre synthesized using Merrifield's solid-phase methodology n an Applied Biosystems 430A peptide synthesizer.

The peptide named 84 TS (MW 2,734) is identical to binding Peptide B described above in Teble 2. The amino acid sequence for this peptide was derived from the trypsin cleavage site of bovine rotavirus VP3 spanning amino acids 231-254 and is as follows: H-Cys-Asn-Ile-Ala-Pro-Ale-Ser-Ile-Val-Ser-Arg-Asn-Iie-Val-Tyr-Thr-Arg-Ale-Gln-Pro-Asn-Gln-Asp-Ile-Ala-OH. The cysteine at position 1 was edded to facilitate coupling to e carrier protein and is not present in the natural sequence. Reevaluetion of the criterie required for binding of Peptide B to VP6-assembled particles enabled the generetion of a shortened version of the binding p ptide which is referred to as SHT (Table 2). The SHT peptide is composed of amino acids 1 and 10-18 from binding Peptide B, plus 2 amino ecids to echieve proper spacing. The amino acid sequence of SHT is as follows: H-Cys-Gly-Ale-Ser-Arg-Asn-Iie-Val-Tyr-Thr-Arg-Ala-OH. The amino ecids glycine (Gly) and alanine (Ale) et positions 2 and 3, respectively, are spacers to distance the cysteine (Cys) from the arginine (Arg).

The remaining three-peptide constructs are composed as follows. Amino acids 1 and 10-18 from Peptide B plus the two spacer amino acids (i.e., SHT) comprise the first 12 amino-terminal amino acids of the construct and the following three amino acid (either Ala-Pro-Ala or Gly-Ala-Pro) are specers which distance th SHT portion of the construct from a specific epitope thet comprises the remaining portion of the peptide construct. Th peptid designated pili-SHT (MW 3,174) has its amin terminal end comprised of the SHT peptide and its carboxy t rminal s quence from the amino terminal region of th F pilin of E. coli. The amino acid sequence of the ntire construct is: H-Cys-Gly-Ala-Ser-Arg-Asn-Iie-Val-Tyr-Thr-Arg-Ala-Ala-Pro-Ale-Gly-Ala-Gly-Ser-Ser-Gly-Gln-Asp-Leu-Met-Ale-Ser-Gly-Asn-Thr-Thr-Val-Ala-OH. The underlined portion indicatas the epitope whose sequence was derived from the F pilin of E. coli, and to which the immune response is to be directed.

The peptide designated Leishmania-SHT (MW 3,876) is comprised of the SHT peptide at the amin terminal end and its carb xy terminal end is derived from a sequence of glycoprot in 63 of th Leishmania donovani. The amino acid sequence of th construct is: H-Cys-Gly-Ala-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ala-Ala-Pro-Ala-Val-Arg-Asp-Val-Asn-Trp-Gly-Ala-Leu Arg-Ile-Ala-Val-Ser-Thr-Glu-Asp-Leu-Lys-Thr-Pro-Ala-Tyr-Ala-OH. Again, th underlining indicates the epitope whose sequence was d rived from glycoprot in 63 of Leishmania donovani and to which the immune response is to be direct d.

The peptide designated BHV-1-SHT (MW 4,645) is comprised of the SHT peptide at the amin terminal, whil its carboxy terminal is derived from an epitope which spans amino acids 323-345 of bovine herpes virus-1 glycoprotein g1 (or gB). Hence, the amino acid sequence of the construct is: H-Cys-Gly-Ala-Ser-Arg-Asn-Ile-Val- Tyr-Thr-Arg-Ala-Gly-Ala-Pro-Glu-His-Thr-Ser-Tyr-Ser-Pro-Glu-Arg-Phe-Gln-Gln-Ile-Glu-Gly-Tyr-Tyr-Lys-Arg-Asn-Met-Ala-Thr-Ala-Ala-OH. The two carboxy terminal alanines are spacers. The underlining again indicates the epitope whose sequence was derived from AA323-345 of BHV-1 glycoprotein and to which the immune response is to be directed. In order to evaluate the level of the immune response induced by this epitope, a larger sequence spanning amino acids 319-352 on bovine herpes virus 1 glycoprotein gI (or gB) was used as the capture antigen in ELISAs, described below. The amino acid sequence of this larger peptide, called BHV-1, is: Gly-Ala-His-Arg-Glu-His-Thr-Ser-Tyr-Ser-Pro-Glu-Arg-Phe-Gln-Gln-Ile-Glu-Gly-Tyr-Tyr-Lys-Arg-Asp-Met-Ala-Thr-Gly-Arg-Arg-Leu-Lys-Glu-Pro-Ala-Glu. The terminal 2 amino acide alanine (Ala) and glutamic acid (Glu) are spacers.

A slightly different approach was used in order to produce a SHT peptide-epitope construct where the epitope was a carbohydrate moiety. A new SHT peptide was prepared having the sequence shown in Table 2, but with the following peptide spacer at the carboxy terinal instead of the tripeptide described above: -Ala-Pro-Ala-Lys-Ala-Lys-Ala-Lys-Ala-OH. This SHT version has MW 2,054. A capsular polysaccharide moiety was isolated from the bacterium Haemophilus pleuropneumoniae, and then oxidized, hydrolyzed and reductively aminated to the SHT peptide. See Altman et al. (1986) Biochem & Cell Biol. 64:707-716; Porter t al. (1986) J. Immunol. 137:1181-1186. Thie provided a carbohydrete-SHT (CHO-SHT) construct. (NOTE: The taxonomists have recommended that the species name Haemmophilus be replaced with the name Actinobacillus.)

C. Formation of VP6 Assembled Particle - Epitope Constructs

In order to generate VP6 assembled particle-peptide complexes containing the SHT peptide and an epitope of protein origin, the VP6 assembled particles and the peptide constructs were mixed together in a retio of 1:10 (w/w), respectively, since this retio produced a complete ladder indicating that most of the potential binding eites on VP6 were occupied by the peptide. However, any retio from 1:1 up to 1:10 would produce laddering of VP6, albeit to different extents. Verification of binding of the peptide construct to VP6 and establishment of the retio of VP6 assembled particle to peptide construct to be used in preparetions for in vivo studies, was by electrophoresis of the preparations on polyacrylamide gels and observation of VP6 ladd ring, a phenomenon dascribed previously. Verification of the immunoreactivity of the peptide haptens was immunoblot-ELISA reactions using antisere epecific fro the protein from which the haptan (epitope) was derived.

In order to generete a VP6 assembled particle-peptide complex where the epitope was a carbohydret , the VP6 assembled particles and CHO-SHT binding to VP6 was by polyecrylamide gel electrophoresis. In this cas , high molecular weight material was observed which represented the VP6-CHO-SHT complex. Verification of the compositions of the high molecular weight complex, observed in a polyacrylamide gel, was by an immunoblot-ELISA reaction using antisera specific for the carbohydrete and antisere specific for th SHT peptide.

D. Immunization and Serological Responses

In general, for immunization trials 1 to 4, groups of 5 to 10 CD-1 mice were inoculated with one of the four VP6 assembled particle-peptide complexes described above according to the experimental designs shown in Tables 5, 6, 7 and 8. The VP6 assembled particle to peptide construct ratio was always 1:10 (w/w), raspectively. The mice used in these experiments were rotavirus-free unless otherwise stated.

The basic immunization schedule was the same for all trials, except number 5. Basically, animals were bled at week 0 and then immunized intramuscularly with 100 ul of the test preparation at week 1 and then again at week 4. In some trials, a third immunization was administered. The type of immunogen and purpose for immunization in each trial is outlined below. Pooled serum samples were obtained from each group of mice on a weekly interval. Assessment of antibody levels specific for the VP6 aseembled particles (spheres) or th peptide constructs (hapten) was by ELISA and is appropriately indicated in Figures 6-8 corresponding to Trials 2 through 4, respectively, while the immunization protocols were shown in Tables 5 through 8, corresponding to Trials 1 through 4, respectively.

Trial 1 (Tabl 5)

The objective of this trial was to evaluat the dose respons to the spherical carrier - 84TS complex using either Freund's adjuvant or dimethedioctodecyl ammonium bromid (DDA) adjuvant, and to investigate the possibility of carrier suppression (dascribed below). The immunogen used for primary and s condary immunization was th spherical carrier-84TS complex. The immunogen used for tertiary immunization to

investigate carrier suppression was the spherical carrier - 272-295 SHT complex.

Table 5 outlines the exp rimental design used to investigate the dose response t the VP6 assembled particle-84 TS peptide complex using either Freund'e edjuvant r dimethyldioctodecyl ammonium bromide (DDA) edjuvant. Thie study also ettempts to investigate the possibility f a carrier suppression phenom non which is a recently recognized immunoregulatory m chanism and has been described in the literature for other carriere currently in use. Basically, carrier suppression occurs when a host is immunized with a hapten conjugated t an immunogenic carrier to which the animal has been previously exposed or immunized. A strong secondary responee is produc d to the carrier, but the host faile to produce antibodies to th link d hapten. In thie experiment, therefore, animals which were immunized twice with the VP6 assembled particle-84TS peptide complex were then reimmunized e third time, et week 19, with the VP6 assembled particle-275-295-SHT peptide complex. The 275-295 peptide sequence was derived from a neutralizing domain on bovine rotavirus VP7 and represents another example of a viral peptide ettached to VP6 aseembled peticles. Tha VP7 sequence is: Pro-Thr-Thr-Ala-Pro-Gin-Thr-Glu-Arg-Met-Met-Arg-Ile-Asn-Trp-Lys-Lys-Trp-Trp-Gin-Val.

The results illustrated that the VP6 assembled particles are effective in inducing high levels of antibody in vivo in both themselves (approximately 6.0-6.5 $\log_{10}$) as well as to the peptide attached to them (approximat ly 5.5-6.0 $\log_{10}$) when a dose equivalent to 1 ug of VP6 assembled particles is administered. In fact, the level of antibody specified for the peptide at this dose was almost identical to a dose equivalent to 10 ug of peptide bound to 10 ug of peptide bound to 1 ug of VP6 essembled paticles and only slightly less than thet using 100 ug peptide bound to 10 ug of VP6 assembled particles. Furthermore, the level of antibody produced to the peptide was significantly higher than that induced by the equivalent amount of unbound or "free" peptid , except et the 100 ug dose of "free" peptide, where an anti-peptide response of 5.5 $\log_{10}$ was observed.

A comparison of antibody levels in sere from animals administered preparations containing either PCA r DDA illustrated that these two adjuvants were equally effective, at least at the two doses inveetigeted. In eddition, no carrier suppression was observed since an antibody responee (approximately 4.5 $\log_{10}$) to peptide 275-295-SHT could be detected et week 20, when the VP6 assembled particle-275-295-SHT peptid complex was administered at week 19.

Table 5

EXPERIMENTAL DESIGN FOR TRIAL I - DOSE RESPONSE TO SPHERICAL CARRIER +/- PEPTIDES + DDA OR FCA ADJUVANTS

# Mice/ Group	Immunization at Weeks 1 and 4 ug Carrier - ug peptide 84TS[a]				Immunization at Week 19 ug Carrier - ug Peptide 275-295-SHT[b]				Adjuvant[c]
5	0.1	-	0	-	0	-	0	-	FCA/FIA
5	1.0	-	0	-	0	-	0	-	FCA/FIA
5	10	-	0	-	0	-	0	-	FCA/FIA
10	0.1	-	1.0	-	0.1	-	1.0	-	FCA/FIA
10	1.0	-	10	-	1.0	-	10	-	FCA/FIA
10	10	-	100	-	10	-	100	-	FCA/FIA
5	1.0	-	0	-	0	-	0	-	DDA
5	10	-	0	-	0	-	0	-	DDA
5	1.0	-	10	-	1.0	-	10	-	DDA
10	10	-	100	-	10	-	100	-	DDA
10	0	-	0	-	0	-	0	-	FCA/FIA
5	1.0 virus		0	-	0	-	0	-	FCA/FIA
5	10	-	0	-	0	-	0	-	FCA/FIA
5	0	-	0	-	0	-	0	-	DDA
5	0	-	1.0	-	0	-	0	-	FCA/FIA
5	0	-	10	-	0	-	0	-	FCA/FIA
5	0	-	100	-	0	-	0	-	FCA/FIA

a The ratio of spherical carrier to peptide construct is 1:10.

b Peptide 275-295 was derived from VP7 of bovine rotavirus and was linked to the carrier via the SHT peptide. The carrier - 275-295-SHT complex was administered at week 19 in order to investigate carrier suppression phenomenon.

c Freund's Complete Adjuvant (FCA) was used for the primary immunization and Freund's incomplete Adjuvant (FIA) was used for the secondary immunization.

DDA - dimethyl dioctodecylammonium bromide is a quaternary amino surfactant which acts as an adjuvant. It was used for both primary and secondary immunization where specified.

Trial 2 (Table 6 Figure 6)

The objective of this trial was to evaluete the dose response to spherical carrier-BHV-1-SHT complex in rotavirus-free and rotavirus-exposed mice, and to investigete the possibility of carrier suppression. The immunogen used for primary and secondary immunization was the spherical carrier-BHV-1-SHT complex. Th immunogen used for tertiary immunization to investigete carrier suppression was the spherical carrier-pilin-SHT complex.

Table 6 outlines the experimental design used to investigete the dose response to the VP6 assembled particle-BHV-1-SHT complex in both rotavirus-free and rotavirus-exposed mice. In e natural situation some animals as well as humans heve e preexisting antibody titer to rotavirus. Therefore, it was important t investigate whether the presence of such antibodies would influence the immune response to th VP6 essembled particle-peptide complex.

Figure 6 illustrates the antibody responses to the VP6 assembled particle (anti-sphere), the BHV-1-SHT peptide (anti-BHV-1-SHT), and to pilin-SHT (anti-pilin-SHT). The latter antibody response was used t investigate the possibility of carrier suppression. Th quantity of peptide in the VP6-assembled BHV-1-SHT peptide preparation edministered to mice is indicated on the top right corner of each pan l. The arrows below the axis indicating weeks denote the time of immunization.

Table 6

EXPERIMENTAL DESIGN FOR TRIAL 2 - DOSE RESPONSE TO SPHERICAL CARRIER + PEPTIDES IN ROTAVIRUS-FREE (RVF) AND ROTAVIRUS-EXPOSED (RVE) MICE

# Mice/ Group	Rotavirus Status of Mice[a]	Immunization at Weeks 1 and 4 ug Carrier - ug peptide BHV-1-SHT[b]			Immunization at Week 19 ug Carrier - ug Peptide pili-SHT[b,c]			Adjuvant[c]
10	RVF	10	-	100	10	-	100	FCA/FIA
10	RVE	10	-	100	10	-	0	FCA/FIA
10	RVF	1	-	10	1	-	100	FCA/FIA
10	RVE	1	-	10	1	-	10	FCA/FIA
10	RVE	0	-	100	0	-	0	FCA/FIA
10	RVE	0	-	10	0	-	0	FCA/FIA
10	RVF	0	-	0	0	-	0	FCA/FIA
10	RVE	0	-	0	0	-	0	FCA/FIA

[a] Rotavirus-free (RVF) and rotavirus-exposed (RVE) mice.

[b] The ratio of carrier to peptide conatruct is 1:10.

[c] The carrier - pili-SHT complex was administered at week 15 in order to investigate the phenomenon of carrier expression.

[d] Freund's Complete Adjuvant (FCA) was used for the primary immunization and Freund's Incomplete Adjuvant (FIA) was used for the secondary immunization.

Figure 6 illustrates thet ther was no significant difference between th level of antibody produced in rotavirus-fr e (RVF) and rotevirus-exposed (RVE) mice to the VP6 assembled particles and the BHV-1-SHT

peptide, even though the RVE mice had an anti-sphere titer of approximately 3 logs at the start of the immunization schedule. The lowest dose tested in this experiment consisted of 10 ug of BHV-1-SHT peptide and 1 ug of VP6 assembled particles. As illustrated in Figure 6, 10 ug of the BHV-1-SHT peptide alone did not induce a detectable antibody response to the peptide, whereas the same quantity of peptide bound to the VP6 assembled particles induced an antibody response of approximetely 5 logs.

Since the carboxy terminal sequence of the BHV-1-SHT peptide was derived from a larger (BHV-1) peptide described above, it was of interest to test the reactivity of the antibodies specific for the BHV-1-SHT peptide with the parent BHV-1 peptide alone. The level of antibody reacting with the BHV-1 peptide gave an indication of the immunogenicity of the carboxy terminal portion of the BHV-1-SHT peptide; the portion containing the epitope to which an immune response was desired. As illustrated in the anti-BH-1 panels of Figure 6, there was a significant antibody response produced against the carboxy terminal portion of the peptide construct; i.e., the BHV-1 peptide.

The carrier suppression phenomenon was also investigated in thie experiment using a different VP6 assembled particle peptide combination than that described in Trial 1. After two immunizations with the VP6 assembled particle-BHV-SHT peptide complex, the VP6 assembled particle-pilin-SHT peptide complex was administered at week 15. As illustrated in Figure 6, previously existing antibodies to the VP6 assembled particle did not affect the production of antibodies to a new peptide (i.e., pilin-SHT) presented on VP6 assembled particles. Furthermore, carrier euppression was not observed in either RVF or RVE mice since antibodies specific for the pilin-SHT peptide were detected (anti-pilin-SHT panel, Figure 6). Antibodies detected to the pilin-SHT prior to immunization at week 15 were due to reaction with the shared amino terminal portion of the peptide constructs (i.e., SHT peptide).

Trial 3 (Table 7 and Figure 7) and Trial 4 (table 8 and Figure 8)

The objectives of these trials were to evaluate the dose response to spherical carrier-Leishmania-SHT (Trial 3) and spherical carrier-pilin-SHT (Trial 4). The immunogens used for primary and secondary immunization were spherical carrier-Leishmanie-SHT or spherical carrier-pilin-SHT complexee.

Tables 7 and 8 outline the experimental design to investigate the dose response to the VP6 assembled particle-leishmanie-SHT peptide complex and to the VP6 assembled particle-pilin-SHT peptide complex, respectively. The antibody response to the VP6 assembled particles and to both the peptide constructs, shown in Figures 7 and 8, illustrate thet the lower quantity of immunogen which eilcits an antibody response in mice after two immunizations is 0.1 ug of VP6 assembled particles bound to 1.0 ug of peptide. In contrast, for both the Leishmanie-SHT (Figure 7) and pilin-SHT peptides (Figure 8), only 100 ug of free peptide was able to ellcit an immune response.

Table 7

EXPERIMENTAL DESIGN FOR TRIAL 3: DOSE RESPONSE TO SPHERICAL CARRIER + LEISHMANIA-SHT PEPTIDE

# Mice/ Group	ug Carrier	-	ug Peptide Leishmania-SHT[a]	Adjuvant[b]
10	10	-	100	FCA/FIA
10	1.0	-	10	FCA/FIA
10	0.1	-	1.0	FCA/FIA
10	0.01	-	0.1	FCA/FIA
10	0	-	100	FCA/FIA
10	0	-	10	FCA/FIA
10	0	-	1.0	FCA/FIA
10	0	-	0.1	FCA/FIA
10	0	-	0	FCA/FIA
10	1.0 rotavirus		0	FCA/FIA

[a] The ratio of spherical carrier to peptide construct is 1:10.

[b] Freund's Complete Adjuvant (FCA) was used for primary immunization and Freund's Incomplete Adjuvant (FIA) was used for secondary immunization.

Table 8

EXPERIMENTAL DESIGN FOR TRIAL 3: DOSE RESPONSE TO SPHERICAL CARRIER + PILIN-SHT PEPTIDE

# Mice/ Group	ug Carrier	-	ug Peptide Pilin-SHT[a]	Adjuvant[b]
10	10	-	100	FCA/FIA
10	1.0	-	10	FCA/FIA
10	0.1	-	1.0	FCA/FIA
10	0.01	-	0.1	FCA/FIA
10	0	-	100	FCA/FIA
10	0	-	10	FCA/FIA
10	0	-	1.0	FCA/FIA
10	0	-	0.1	FCA/FIA
10	0	-	0	FCA/FIA
10	1.0 rotavirus		0	FCA/FIA

[a] The ratio of spherical carrier to peptide construct is 1:10.

[b] Freund's Complete Adjuvant (FCA) was used for primary immunization and Freund's Incomplete Adjuvant (FIA) was used for secondary immunization.

Trial 5 (Table 9 and Figure 9)

The objective of this trial was to evaluate in swine the dose response to spherical carrier-CHO-SHT complex. The immunogen used for primary and secondary immunization was the spherical carrier-CHO-SHT complex.

In order to test the VP6 assembled particle-CHO-SHT complex, 16 pigs were randomized into 4 groups of 4 pigs each. One group of pigs was left as unvaccinated controls. The other three groupe were immunized with different doses of this preparation as shown in Table 9 and according to the following immunization schedule.

Table 9

EXPERIMENTAL DESIGN FOR TRIAL 5: MEASURING SWINE ANTIBODIES TO SPHERICAL CARRIER + CARBOHYDRATE-PEPTIDE (CHO-SHT

# Pigs/ Group	ug Carrier-ug CHO-SHT[a]	Adjuvant[b]
4	1.0 - 0.1	marcol 52
4	10 - 1.0	marcol 52
4	100 - 10	marcol 52
4	0 - 0	marcol 52

[a] The ratio of carrier to CHO-SHT is 10:1.

[b] Marcol 52 - an oil-based.

Immunization Schedule

Weeks	Procedure
0	randomize 16 pigs into 4 groups and bleed
1	vaccinate intramuscularly, left neck, 2 ml dose
3	bleed, boost intramuscularly, right neck, 2 ml dose
4	bleed
5	bleed

The antibody reeponses to the carbohydrete moiety were determined by ELISA and are ehown in Figure 9. both 1.0 ug of CHO-SHT bound to 10 ug of VP6 assembled particles (carrier) and 10 ug of CHO-SHT bound to 100 ug of VP6 assembled particles induce an immune response which was significantly higher than thet detected in animals given marcol 52 edjuvant alone or 0.1 ug of CHO-SHT bound to 1.0 ug of VP6 assembled particias.

8. Covalent Coupling of Haptens to VP6

The peptide designeted FMDV-SHT is comprised of the SHT peptide at the amino terminal end. The amino ecid sequence of the construct is: H-Cys-Gly-Ala-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ale-Gly-Als-Gly-Val-Pro-Asn-Leu-Arg-Gly-Asp-Leu-Gin-Val-Leu-Ale-Gin-Lys-Val-Ale-Arg-Thr-Ale-Ala-OH. The underlining indicat s the epitope whose sequence was derived from e sequence from protein VP1 of the O_1 Kaufbeuren strain of foot and mouth disease (O_1 K FMDV).

The FMDV portion of the above peptide plus the C terminal specer, thet is H-Val-Pro-Asn-Leu-Arg-Gly-Asp-Leu-Gin-Val-Leu-Ale-Gin-Lys-Val-Ale-Arg-Thr-Ala-Ala-OH, was also synthesized, this underlining indicatee the specar. This peptide without the SHT sequence (FMDV) was then chemically coupled using 1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimide HCl in carbonete buffer pH 9.0 to VP6 spheres reassembl d (described previ usly) for 4-8 hrs. The VP6 spheres with the peptide bonded to them were isoleted from the reaction

mixture by ultracentrifugation on e cesium chloride gradient. The product was recovered at a density approximately qual to that of the reassembled spheres.

This preparati n was then used to immunize groups of mice. Wh n used with Freund' Complete Adjuvant, the groups which were given 10 or 100 ug per mouse reeponded with anticarrier antibodies and the mice given 100 ug/mouse responded with antipaptide to a titer of $1/10^3$. This shows peptides or oth r molecules can be covalently attach d through one of several possibi activating reactions to VP6 spheres without th use f a binding peptide. This alternate method of ettachment to the VP6 spheres does not interfere with the production of antibodies to these haptenic molecules.

The foregoing examples provide specific embodiments of the present invention, other embodiments b ing readily within the ekill of the art. Thue, the scope of the present invention is defined by the following claims without limitation to the foregoing examples.

Claims

1. A composition capable of raising an immunological response in a mamal to a selected epitope comprising an immunological carrier complex, said complex comprised of an epitope-bearing molecule expressing said epitope of interest selected from the group consisting of polypeptides, carbohydretes and nucleic acids; said epitope-bearing molecule being coupled to a carriar protein selected from th grop consisting of monomers and oligomers of e polypeptide homologous to e rotavirus VP6 inner capsid protein amino acid sequence.

2. The composition of claim 1 wherein said eiptope-bearing molecule ie e polypeptide.

3. The composition of claim 1 or claim 2 wherein said carrier protein is said VP6 inner cepsid protein.

4. The composition of any one of claims 1 to 3 wherein said carrier protein is an oligomer in the form of a particle.

5. The composition of claim 4 wherein said carrier protein is a spherical particle or a tubular particle.

6. The composition of any one of claims 1 to 5 wherein said coupling of said carrier protein and said epitope-bearing molecule is through e protein-protein interaction.

7. In e vaccine composition wherein the epitope of interest ie on e polypeptide bound to a carrier protein, the improvement comprising using rotavirus VP6 inner capside polypeptides as said carrier protein.

8. The vaccine composition of claim 7 wherein said molecule bearing the eiptope of interest is bound to said carrier protein through e protein-protein interaction between said carrier protein and an amino ecid sequence linked in said molecule.

9. A composition according to claim 6 of claim 8 wherein said amino acid sequence is selected from the group consisting of:

(a)
Cys-Asp-gly-Lys-Tyr-Phe-Ale-Tyr-Lys-Val-Glu-Thr-ile-Leu-Lys-Arg-Phe-His-Ser-Met-Tyr-Gly;

(b)
Cys-Asn-Ile-Ale-Pro-Ala-Ser-Ile-Val-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ale-Gln-Pro-Asn-Gln-Asp-Ile-Ala; and

(c) an amino acid sequence comprised of fragments of said saquences (a) or (b), any deletions or substitutions being selected to maintain tha ability to bind to said carrier protein.

10. A composition according to claim 9 wherein said amino acid sequence (c) is selected from the group consisting of:

(i)
Cys-Gly-Ala-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ala;

(ii)
Cye-Cly-Ala-Ser-Ser-Asn-Ile-Val-Tyr-Thr-Arg-Ale; and

(iii)
Asp-Thr-Phe-Glu-Gly-Ale-Pro-Ala-Pro-Ale-Cys-Gly-Ala-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ala.

11. The use of a composition as defined in claim 1 or claim 7 in preparing a vaccine by providing a composition that is effective in raising neutralizing antibodies to said selected epitope in e mammal.

12. A method of forming e vaccine composition for e selected epitope comprising:

(a) providing an immunologic carrier protein selected from the group consisting of monomers and oligomers of a polypeptide homologous to a rotavirus VP6 inner capsid protein amino ecid sequence;

(b) providing an epitope-bearing molecule expressing said selected epitope, said epitope bearing molecule being eelected from tha group consisting of polypeptides, carbohydretes and nucl i acids; and

(c) contacting said carrier protein and said pitope-bearing molecul und r c nditions whereby said epitope-bearing molecule becomes bound to said carrier protein.

13. The us of e protein selected from the group consisting of monomers and oligomers of a polypeptide homologous to e rotavirus VP6 inner capsid protein amino acid sequence, e.g., a rotavirus VP6 inner capsid protein, in preparing a medicament or vaccine.

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FIG. 1

MET ASP VAL LEU TYR SER LEU SER LYS THR LEU LYS ASP ALA 14
5'- GGCTTTTAAACGAAGTCTTCAAC ATG GAT GTC CTA TAC TCT TTG TCA AAG ACT CTT AAA GAC GCT 65

ARG ASP LYS ILE VAL GLU GLY THR LEU TYR SER ASN VAL SER ASP LEU ILE GLN GLN PHE 34
AGA GAC AAA ATT GTC GAA GGC ACA TTG TAT TCT AAC GTG AGT GAT CTA ATT CAA CAA TTT 125

ASN GLN MET ILE ILE THR MET ASN GLY ASN GLU PHE GLN THR GLY GLY ILE GLY ASN LEU 54
AAT CAA ATG ATA ATT ACT ATG AAT GGA AAT GAA TTT CAA ACT GGA GGA ATC GCT AAT TTG 185

PRO ILE ARG ASN TRP ASN PHE ASN PHE GLY LEU LEU GLY THR THR LEU LEU ASN LEU ASP 74
CCA ATT AGA AAC TGG AAT TTT AAT TTC GGG TTA CTT GGA ACA ACT TTG CTG AAC TTA GAC 245

ALA ASN TYR VAL GLU THR ALA ARG ASN THR ILE ASP TYR PHE VAL ASP PHE VAL ASP ASN 94
GCT AAT TAT GTT GAA ACG GCA AGA AAT ACA ATT GAT TAT TTC GTG GAT TTT GTA GAC AAT 305

VAL CYS MET ASP GLU MET VAL ARG GLU SER GLN ARG ASN GLY ILE ALA PRO GLN SER ASP 114
GTA TGC ATG GAT GAG ATG GTT AGA GAA TCA CAA AGG AAC GGA ATT GCA CCT CAA TCA GAC 365

SER LEU ARG LYS LEU SER ALA ILE LYS PHE LYS ARG ILE ASN PHE ASP ASN SER SER GLU 134
TCC CTA AGA AAG CTG TCA GCC ATT AAA TTC AAA AGA ATA AAT TTT GAT AAT TCG TCG GAA 425

TYR ILE GLU ASN TRP ASN LEU GLN ASN ARG ARG GLN ARG THR GLY PHE THR PHE HIS LYS 154
TAC ATA GAA AAC TGG AAT TTG CAA AAT AGA AGA CAG AGG ACA GGT TTC ACT TTT CAT AAA 485

PRO ASN ILE PHE PRO TYR SER ALA SER PHE THR LEU ASN ARG SER GLN PRO ALA HIS ASP 174
CCA AAC ATT TTT CCT TAT TCA GCA TCA TTT ACA CTA AAT AGA TCA CAA CCC GCT CAT GAT 545

ASN LEU MET GLY THR MET TRP LEU ASN ALA GLY SER GLU ILE GLN VAL ALA GLY PHE ASP 194
AAT TTG ATG GGC ACA ATG TGG TTA AAC GCA GGA TCG GAA ATT CAA GTC GCT GGA TTT GAC 605

TYR SER CYS ALA ILE ASN ALA PRO ALA ASN ILE GLN GLN PHE GLU HIS ILE VAL PRO LEU 214
TAC TCA TGT GCT ATT AAC GCA CCA GCC AAT ATA CAA CAA TTT GAG CAT ATT GTG CCA CTC 665

ARG ARG VAL LEU THR THR ALA THR ILE THR LEU LEU PRO ASP ALA GLU ARG PHE SER PHE 234
CGA AGA GTG TTA ACT ACA GCT ACG ATA ACT CTT CTA CCA GAC GCG GAA AGG TTT AGT TTT 725

PRO ARG VAL ILE ASN SER ALA ASP GLY ALA THR THR TRP PHE PHE ASN PRO VAL ILE LEU 254
CCA AGA GTG ATC AAT TCA GCT GAC GGC GCA ACT ACA TGG TTT TTC AAC CCA GTG ATT CTC 785

ARG PRO ASN ASN VAL GLU VAL GLU PHE LEU LEU ASN GLY GLN ILE ILE ASN THR TYR GLN 274
AGG CCG AAT AAC GTT GAA GTG GAG TTT CTA TTG AAT GGA CAG ATA ATA AAC ACT TAT CAA 845

ALA ARG PHE GLY THR ILE VAL ALA ARG ASN PHE ASP THR ILE ARG LEU SER PHE GLN LEU 294
GCA AGA TTT GGA ACT ATC GTA GCT AGA AAT TTT GAT ACT ATT AGA CTA TCA TTC CAG TTA 905

MET ARG PRO PRO ASN MET THR PRO ALA VAL ALA VAL LEU PHE PRO ASN ALA GLN PRO PHE 314
ATG AGA CCA CCA AAC ATG ACA CCA GCA GTA GCA GTA CTA TTC CCG AAT GCA CAG CCA TTC 965

GLU HIS HIS ALA THR VAL GLY LEU THR LEU ARG ILE GLU SER ALA VAL CYS GLU SER VAL 334
GAA CAT CAT GCA ACA GTG GGA TTG ACA CTT AGA ATT GAG TCT GCA GTT TGT GAG TCT GTA 1025

LEU ALA ASP ALA SER GLU THR LEU LEU ALA ASN VAL THR SER VAL ARG GLN GLU TYR ALA 354
CTC GCC GAT GCA AGT GAA ACT CTA TTA GCA AAT GTA ACA TCC GTT AGG CAA GAG TAC GCA 1085

ILE PRO VAL GLY PRO VAL PHE PRO PRO GLY MET ASN TRP THR ASP LEU ILE THR ASN TYR 374
ATA CCA GTT GGA CCA GTC TTT CCA CCA GGT ATG AAC TGG ACT GAT TTA ATC ACC AAT TAT 1145

SER PRO SER ARG GLU ASP ASN LEU GLN ARG VAL PHE THR VAL ALA SER ILE ARG SER MET 394
TCA CCG TCT AGG GAG GAC AAT TTG CAA CGC GTA TTT ACA GTG GCT TCC ATT AGA AGC ATG 1205

LEU ILE LYS ??? 397
CTC ATT AAA TGA GGACCAAGCTAACAACTTGGTATCCAACTTTGGTGAGTATGTAGCTATATCAAGCTGTTTGAA 1280

CTCTGTAAGTAAGGATGCGTATACGCATTCGCTACACTGAGTTAATCACTCTGATGGTATAGTGAGAGGATGTGACC-3' 1357

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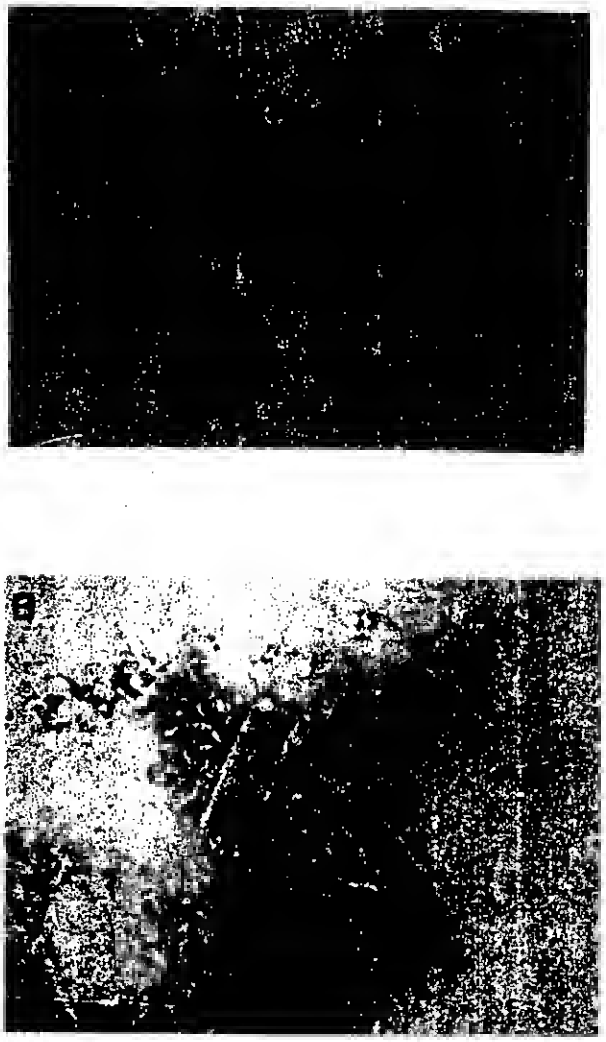
FIG. 2



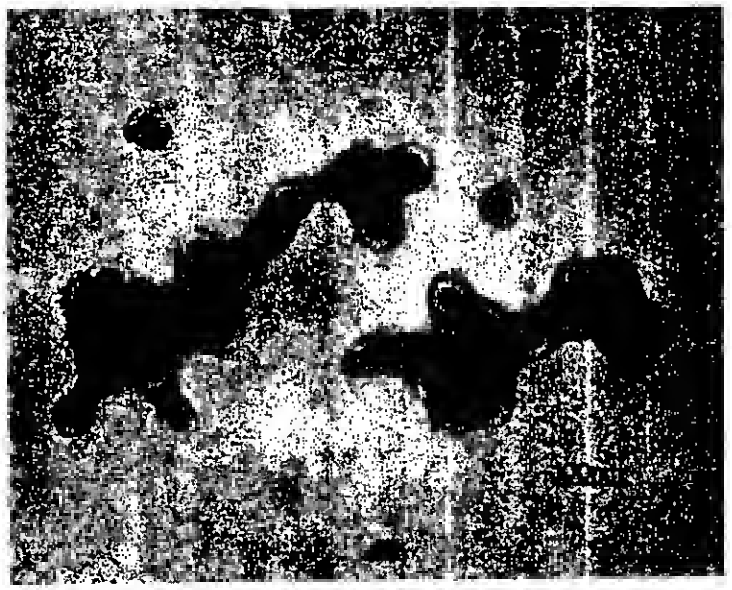


FIG. 3

FIG. 4

FIG. 5

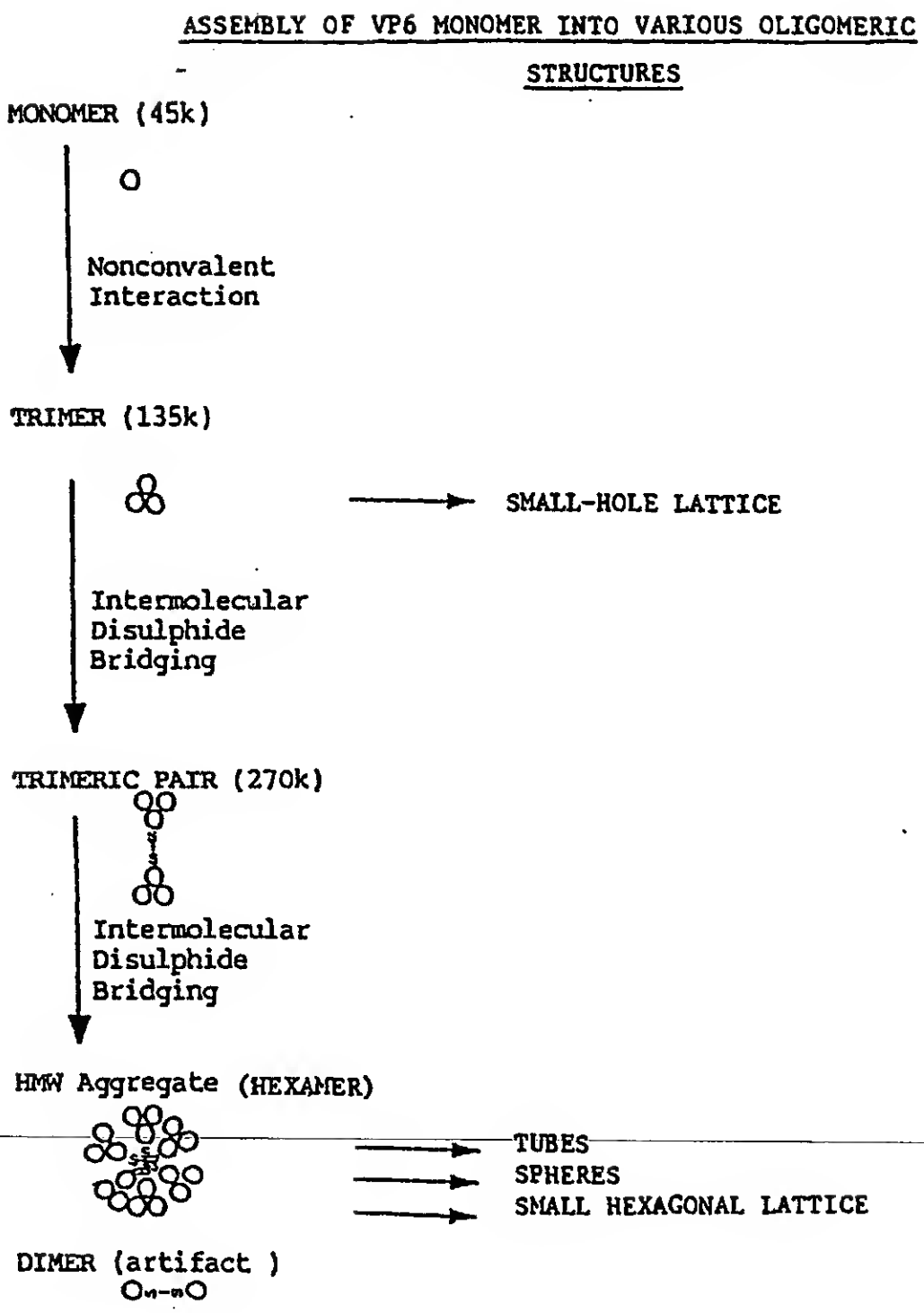

ASSEMBLY OF VP6 MONOMER INTO VARIOUS OLIGOMERIC STRUCTURES
MONOMER (45k)
Nonconvalent Interaction
TRIMER (135k)
SMALL-HOLE LATTICE
Intermolecular Disulphide Bridging
TRIMERIC PAIR (270k)
Intermolecular Disulphide Bridging
HMW Aggregate (HEXAMER)
TUBES
SPHERES
SMALL HEXAGONAL LATTICE
DIMER (artifact)

FIG. 6

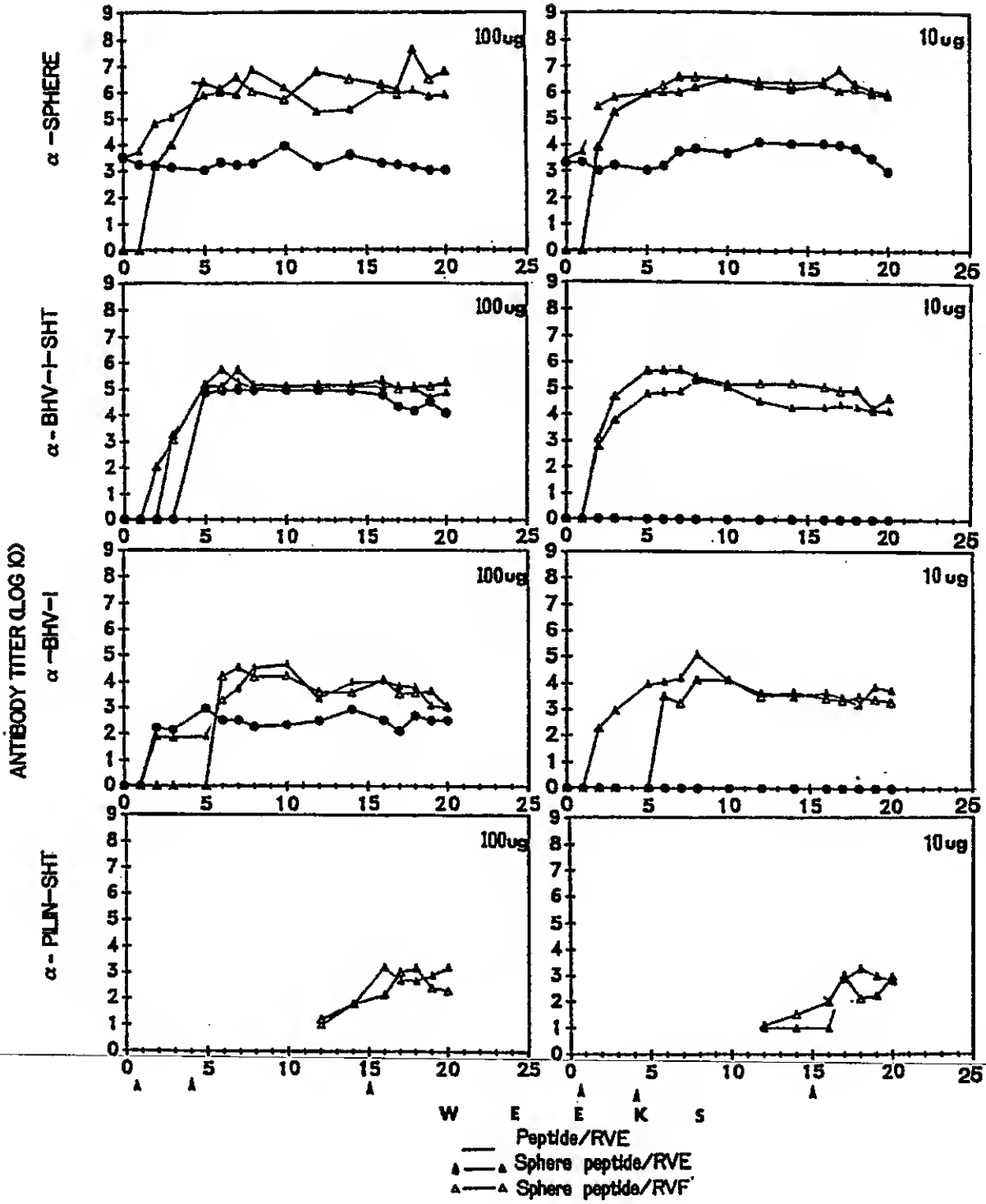

ANTIBODY TITER (LOG 10)
α -SPHERE
α - BHV-1-SHT
α -BHV-1
α - PLN-SHT
100ug
10ug
WEEKS
Peptide/RVE
Sphere peptide/RVE
Sphere peptide/RVF

FIG. 7

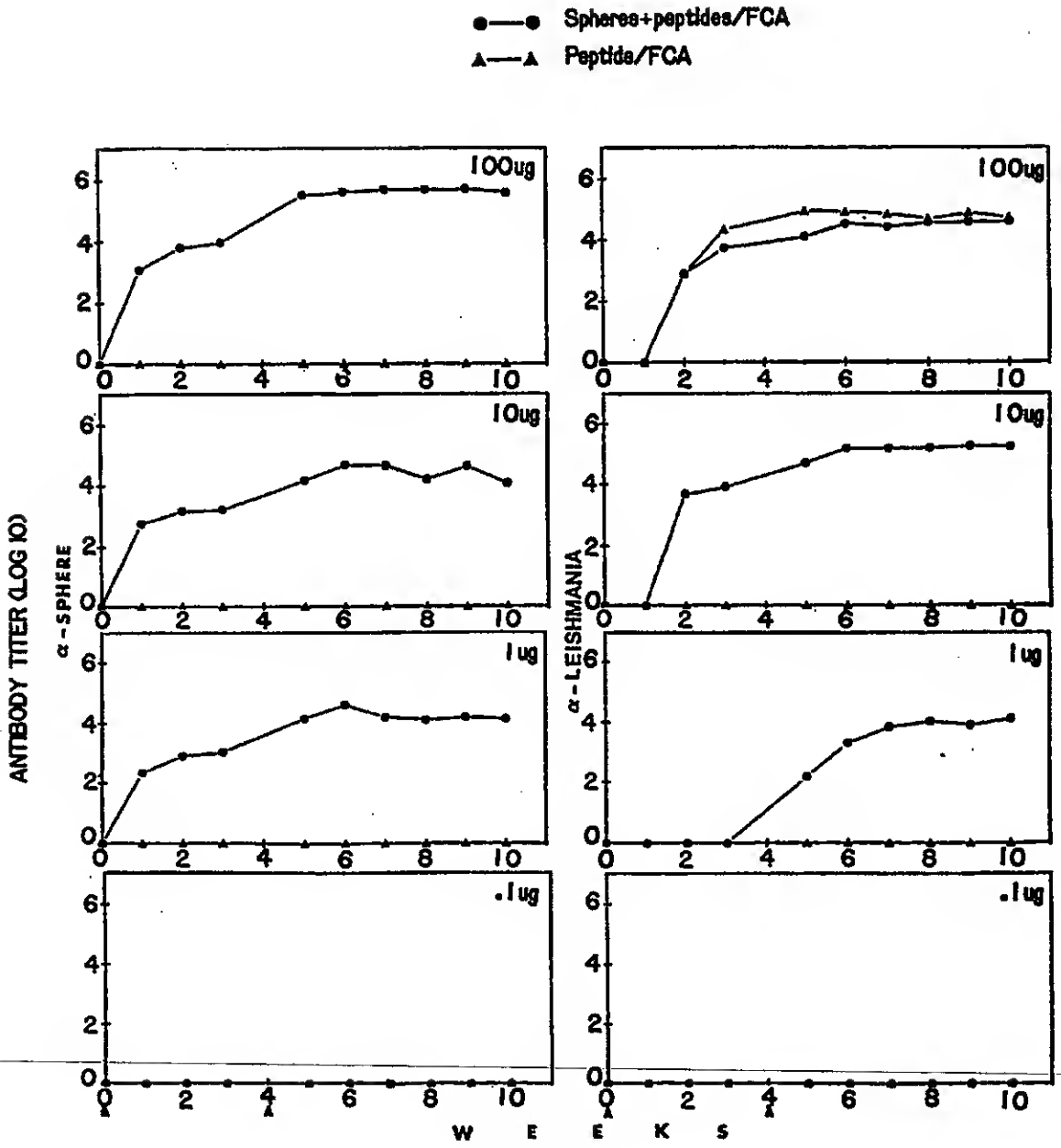

●—● Spheres+peptides/FCA
▲—▲ Peptides/FCA
100ug
10ug
1ug
.1ug
α-SPHERE
α-LEISHMANIA
ANTIBODY TITER (LOG 10)
WEEKS

FIG. 8

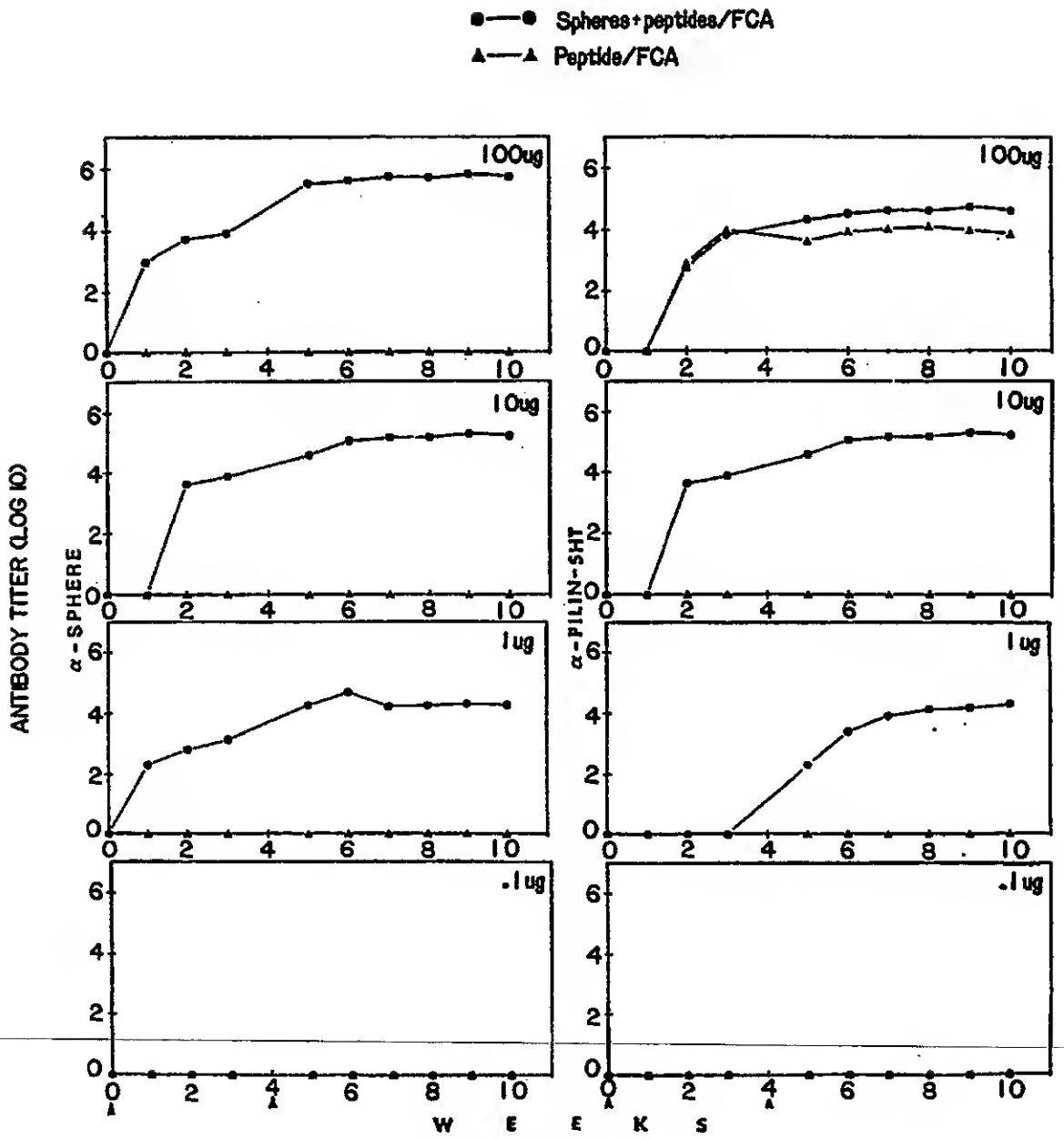
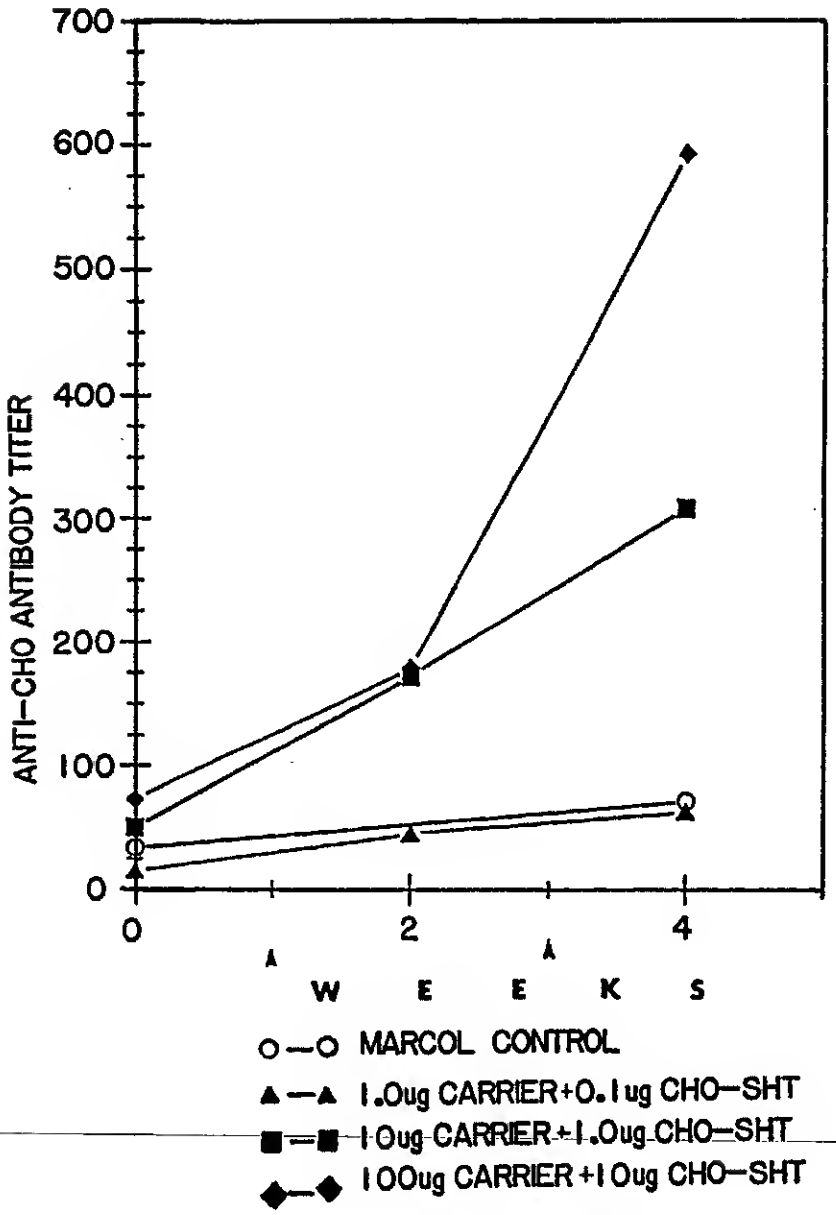

●—● Spheres+peptides/FCA
▲—▲ Peptide/FCA
ANTIBODY TITER (LOG 10)
α-SPHERE
α-PILIN-SHT
100ug
10ug
1ug
.1ug
0
2
4
6
8
10
WEEKS

FIG. 9


700
600
500
400
300
200
100
0
ANTI-CHO ANTIBODY TITER
0
2
4
WEEKS
O—O MARCOL CONTROL
▲—▲ 1.0ug CARRIER+0.1ug CHO-SHT
■—■ 10ug CARRIER+1.0ug CHO-SHT
◆—◆ 100ug CARRIER+10ug CHO-SHT